Pulp reactions in replanted and autotransplanted teeth of dogs

by
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PULP REACTIONS IN REPLANTED AND AUTOTRANSPLANTED TEETH OF DOGS

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Abstract In order to obtain more knowledge of the pulp reactions after tooth replantation and autotransplantation it was considered of interest to study in immature teeth: 1. the revascularization of the pulp by means of a microangiographic technique, 2. the vitality of the pulp during and after the period of pulpal revascularization by histochemical demonstration of oxido-reductase activity and 3. possible tissue changes in the pulp with histologic techniques. Further, it was intended to study in mature teeth: 1. the effect of apicoectomy on the revascularization capacity of the pulp by a microangiographic technique, and 2. the effect of apicoectomy on the tissue reactions of the pulp with histologic techniques. The experimental material consisted of 181 teeth, and 116 of these teeth had partly formed roots at the time of the operation, while 65 teeth had fully formed roots. The observation periods ranged from 1 to 180 days. The following observations were made:

- The pulp of all tooth grafts as a rule initially became necrotic.
- Immature teeth showed pulpal repair with a cell-rich connective tissue that later in part was replaced by hard tissue.
- Mature teeth, that were replanted or autotransplanted without apicoectomy did not exhibit pulpal repair.
- Apicoectomized mature teeth showed pulpal repair as a rule. The repair process was similar to that seen in immature teeth, but proceeded in a slower rate.
- External root resorption had affected all teeth, and repair of the resorption cavities had usually occurred simultaneously with the repair of the pulp.
- Repair of external resorption cavities was also noted in teeth with necrotic pulp tissue, indicating that there are other factors operating in the resorption process.

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V. Skoglund, A.: Pulpal changes in replanted and autotransplanted apicoectomized mature teeth of dogs. Submitted for publication, Int. J. Oral Surg.

The papers will be referred to by their Roman numerals.

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Replantation and autotransplantation of teeth, which have been carried out since ancient times, have attracted increasing interest especially during this century, and a great number of case reports have been published. (For review of the literature see Widman 1915, Nordenram 1963, Öhman 1965, Natiella, Armitage and Greene 1970, Oksala 1974, Heithersay 1976). The prognosis of such transplantation procedures is determined by many factors, such as the degree of root development at the time of the accident or operation, immature teeth being subjected to pulpal repair more often than mature teeth, the extent and type of trauma at the accident or operation, the extraalveolar or extraoral time, and the storage during the extraoral time (Löe and Waerhaug 1961, Knight, Gans and Callandra 1964, Deeb, Prietto and McKenna 1965, Öhman 1965, Andreasen and Hjørting-Hansen 1966a, Anderson, Sharav and Massler 1968, Sherman 1968, Andreasen, Hjørting-Hansen and Jølst 1970, Bolton 1974, Çvek, Granath and Hollender 1974, Andreasen, Reinholdt, Riis, Dybdahl, Söder and Otteskog 1978).

An early postoperative infection is known to interfere seriously with the repair of the periodontal membrane (Singh and Dudani 1970, Moss 1975). Other complications that might occur are root resorptions and pulp necrosis. It is believed that surface resorption is caused by a localized injury to the periodontal membrane or cementum (Andreasen and Hjørting-Hansen 1966b). This type of resorption heals spontaneously. A more extensive injury, such as excessive scraping or drying of the root during the extraoral time, may give rise to external replacement resorption (Löe and Werhaug 1961, Deeb et al. 1965, Çvek et al. 1974). External inflammatory resorption, according to Andreasen and Hjørting-Hansen

(1966b), is resorption of both cementum and dentin, seen together with inflammation of the adjacent periodontal tissue. It has been suggested that such resorption is caused by invasion of autolyzed necrotic pulp tissue into the dentinal tubules (Andreasen 1972). This view is supported by the finding that proper endodontic treatment may lead to arrest of the resorption process (Andreasen 1971, Çvek 1973). It should, therefore, be possible to prevent the establishment or progression of inflammatory root resorption by endodontic treatment as a routine procedure, either performed at the time of the operation (Hovinga 1969) or a short time after the operation (Andreasen and Hjørting-Hansen 1966a, Andreasen et al. 1970). Repair of the pulp, on the other hand, should make endodontic treatment unnecessary.

A satisfactory nutritional system is a prerequisite for tissue vitality. Little is known, however, from previous investigations, about the vascular system and the metabolism of the pulp after tooth replantation and autotransplantation.

Although pulpal repair often has occurred in immature replanted and autotransplanted teeth, these have been frequently subjected to some undesirable late pulpal changes such as internal resorption and obliteration of the root canal (Nordenram 1963, Öhman 1965, Lovius, Atherton and Wynne 1972, Oksala and Kallionemi 1977, Slagsvold and Bjercke 1978). Pulpal obliteration has been interpreted as a sign of pulpal vitality and has been considered a positive reaction (Nordenram 1963, Lovius et al. 1972). It should, however, be borne in mind that obliterated teeth might necrotize (Andreasen et al. 1970), and that endodontic treatment in such teeth often is complicated or even infeasible. Other reactions that might be related to changes in the condition of the pulp are disturbed and decreased root growth (Slagsvold and Bjercke 1974). It would, thus, be a great advantage if such pulpal changes could be avoided.

Different opinions exist in the literature as to whether the pulp of replanted and autotransplanted mature teeth has a reasonable high repair capacity or not. As examples of conflicting results in previous studies, it can be mentioned that Moss (1968) and Thonner and Meijer (1969) have reported signs of pulpal vitality in a majority of the mature replanted and autotransplanted human teeth in their material, while Widman (1915) and Hasselgren, Larsson and Rundquist (1977) have demonstrated pulp necrosis.

In an attempt to improve the repair capacity of the pulp, apicoectomy has been carried out in some experiments as a routine procedure at the time of replantation and autotransplantation of mature teeth (Flath 1963, Kosnevish 1974, Walsh, Kafrawy and Roche 1978). Further, some investigators have accidentally fractured a curved root tip at the time of the operation, and such fracturing of the root has also been performed deliberately to simplify the operative procedures (Bowdler 1955, Bolton 1974, Oksala 1974, Kallionemi and Oksala 1977). It has, however, not been possible to ascertain whether fracturing of the root tip or apicoectomy improves the repair capacity of the pulp. In a clinical and radiographic follow-up study Flath (1963) has detected signs of pulpal vitality in most teeth subjected to such treatment, a finding which was supported by the results of Kosnevish (1974) in an experimental investigation on dogs. Kallionemi and Oksala (1977), on the other hand, reported contradictory results in their clinical study.

Purpose of the present investigations

In order to obtain more knowledge of the pulp reactions after tooth replantation and autotransplantation it was considered of interest to study in immature teeth:

I. the revascularization process of the pulp by means of a microangiographic technique,

II. the vitality of the pulp during and after the period of pulpal revascularization by histochemical demonstration of oxido-reductase activity,

III. possible tissue changes in the pulp during and after the period of pulpal revascularization with the use of histologic techniques.

Further it was intended to study in mature replanted and autotransplanted teeth:

IV. the effect of apicoectomy on the revascularization capacity of the pulp by a microangiographic technique, and

V. the effect of apicoectomy on the tissue reactions of the pulp with histologic techniques.

MATERIAL

The experimental material consisted of 181 single-rooted teeth from 20 mongrel dogs 4 months to 5 years old. Five teeth, that were exfoliated during the first postoperative days, are not included in this material. One hundred and sixteen of the 181 teeth had partly formed roots at the beginning of the experiment (I,II,III), and the remaining 65 teeth had fully formed roots (IV,V).

METHODS

Replantation and autotransplantation procedures

The dogs were anesthetized by intravenous administration of a barbiturate solution (Penthotal sodium®, Abbott, Brussels, Belgium), and the experimental teeth were extracted. During the extraoral time, that did not exceed 10 minutes, the teeth were stored in sterile isotonic saline solution at room temperature. The teeth were then replanted or autotransplanted to the socket of the contralateral tooth.

Penicillin (Suspenin Transin ®, Kabi, Stockholm, Sweden, or Penicillin prokain Vet. ®, Novo, Copenhagen, Denmark) (300.000-600.000 I.U. per day) was given intramuscularly the day before the operation and the following seven days or until the time of sacrifice. The animals were put on a diet of soft food for 2 weeks after the transplantation, or until death.

Apicoectomy

Apicoectomy was performed by means of a surgical wire cutter coronally of the delta-formed apical portion of the root canal of mature teeth of dogs (IV,V). About one fourth of the length of the root was removed.

Observation periods

The observation periods ranged from 1 to 180 days (I,II, III) and from 10 to 120 days (IV,V).

Microangiographic and histologic technique

At the end of the observation periods the animals were anesthetized by intravenous administration of a barbiturate solution (Mebumal Vet. ®, ACO, Solna, Sweden) and Heparin ® (Vitrum, Stockholm, Sweden) was given intramuscularly, 500 I.U. per kilogram of body weight (I,III,IV,V). The common carotid arteries were dissected free and canalized. A warm (37°C) barium sulfate injection medium consisting of Micropaque ® (Nicholas, Slough, Bucks, England) (60 percent) and saline (40 percent) was injected into the arteries by means of a pump at a pressure slightly above the systolic blood pressure. The jugular veins were cut. At the end of the injection a mixture of Micropaque ® (60 percent) and 4 percent neutral buffered

formaldehyde (40 percent) was used and the dogs were sacrificed during this procedure. The jaws were dissected free, fixed in 4 percent formaldehyde and demineralized in nitric acid.

In the experiments reported in papers I and IV about 1 mm thick longitudinal sections were cut from the middle portion of the teeth in a bucco-lingual direction. These slices were dehydrated and embedded in paraffin. Micro-radiographs were then made with a Philips diffraction generator and a diffraction tube on Kodak high-resolution plates or spectroscopic plates 649-0.

In the experiments reported in papers III and V, tissue blocks, each containing one tooth, were dehydrated and embedded in paraffin. Longitudinal serial 4-7 µm thick sections were made from the teeth in a bucco-lingual direction. These sections were stained with hematoxylin and eosin or according to the van Gieson or Brown and Brenn methods.

Enzyme histochemical techniques

The dogs were anesthetized and sacrificed by an overdose of a barbiturate solution (Mebumal Vet.®, ACO, Solna, Sweden). The jaws were then immediately excised and frozen in hexane cooled with solid CO_2 ($-75^{\circ}C$). While still frozen the jaws were divided into pieces containing one to three teeth. These pieces were embedded in an aqueous solution of carboxymethyl cellulose, and 20 µm thick longitudinal sections were made in a bucco-lingual direction at $-18^{\circ}C$. The sections were freeze-dried for at least 48 hours and then transferred to room temperature in an airtight box in order to protect the tissues from condensation of moisture. Sections attached to the tape were then incubated for histochemical demonstration of succinate dehydrogenase (E.C.1.3.99.1), lactate dehydrogenase (E.C.1.1.1.27), glucose-6-phosphate dehydrogenase (E.C.1.1.49) and cytochrome oxidase (E.C.1.9.31). The incubations for the three dehydrogenases were carried

out at 37°C as described by Barka and Anderson (1963), and the incubations for cytochrome oxidase were performed according to Seligman, Karnovský, Wasserkrug and Hanker (1968).

RESULTS

Immature replanted and autotransplanted teeth

The pulp was completely devoid of contrast-filled blood vessels 1 day after the operation (I). At this observation time and also 2 days postoperatively staining for the four oxido-reductases was demonstrated in the whole pulp cavity, and the staining intensity was comparable to that of intact teeth (II). After 4 days the foraminal area of the pulp showed a high staining intensity (II), and a well vascularized, cell-rich connective tissue was observed apically (III). Further coronally the tissue was only weakly stained and small, roundish cell-nuclei were seen (II,III). In a few teeth, at this observation time, contrast-filled blood vessels were detected in the whole root pulp and in areas of the coronal pulp (I,III), i.e. in weakly stained tissue. Contrast-filled blood vessels were found in the apical half of the pulp 10 days post-operatively (I). It was shown that such vessels reached from a well-stained, cell-rich connective tissue in the apical region into weakly stained tissue further coronally (III). In the border zone between vascularized and non-vascularized tissue large accumulations of extravasated erythrocytes and some inflammatory cells were evident (III). After 30 days the whole pulp showed contrast-filled blood vessels in a majority of the teeth (I). At this observation time staining for the four oxido-reductases was demonstrated in all areas (II), and a cell-rich, well vascularized connective tissue was observed in the main part of the root canal (III). A cell-containing hard tissue had formed on the root canal walls in most of the teeth and in some teeth also centrally in the pulp.

A slight amount of dentin had been laid down in the apical part of the pulp. After 180 days the pulp tissue was usually reduced in the number of cells and blood vessels as compared with the findings after 30 days (I, III), and large areas of the pulp were usually occupied by a cell-containing, atubular hard tissue that resembled bone or cementum, and which in some of the teeth was continuous with the alveolar bone (III). In a few teeth a normal pulp tissue was observed with an intact odontoblastic layer. A normal amount of tubular dentin had formed postoperatively on the root canal walls in these teeth, and cell-containing hard tissue was not found in the pulp cavity. Root resorption had affected all teeth and repair of the resorption lacunae had occurred through apposition of a cell-containing hard tissue.

Mature replanted and autotransplanted teeth

A majority of the nonapicoectomized teeth did not show pulpal revascularization (IV), and a weakly stained, nonvascularized tissue with only vaguely discernible tissue structures was evident throughout the pulp (IV,V). In one nonapicoectomized 120 day-tooth a few bacteria were seen in the necrotic tissue (V). External root resorption had affected all teeth and repair of the resorption cavities was often seen after 120 days although pulpal repair had not occurred.

In most of the apicoectomized teeth contrast-filled blood vessels were found 10 days postoperatively in the apical part of the pulp (IV) and a cell-rich connective tissue occupied this area (V). Further coronally the normal morphologic structure of the tissue was only vaguely discerned and small roundish cell-nuclei were seen. Half or more of the pulp was usually revascularized 30 days postoperatively (IV), and a cell-rich and well-stained tissue was noted in the vascularized areas (V).

The nonvascularized parts of the pulp showed a weakly stained tissue and lack of the normal morphologic architecture. In the border zone between vascularized and nonvascularized areas accumulations of extravasated erythrocytes and some inflammatory cells were seen. After 120 days, most of the apicoectomized teeth exhibited contrast-filled blood vessels (IV), and a well-stained connective tissue was evident throughout the pulp in a majority of the teeth (V). A cell-containing hard tissue that resembled bone or cement had formed on the root canal walls in all teeth, and was also observed centrally in the pulp in some teeth. Advanced internal resorption was seen in one tooth, which showed severe inflammation. External root resorption had affected all teeth and repair of the resorption cavities had usually occurred after 120 days.

DISCUSSION

In the investigations reported in papers I and IV, the revascularization process of the pulp of replanted and autotransplanted teeth was studied with a microangiographic technique, adopted from Bell (1969) and slightly modified. It has been shown that injection methods are suitable in revascularization research (Clemmesen 1967, Haller and Billingham 1967, Bell 1969, Smahel and Ganzoni 1970, Alm and Strömberg 1974, Östrup and Fredricksen 1974). With microangiography a more perspicuous view of the vasculature can be obtained than with routine histologic techniques. Moreover, only vessels involved in the circulation are registered. However, the entire microvascular anatomy cannot constantly be visualized, mainly due to physiologic and technical variations (Rubin 1964, Lundskog, Brånemark and Lindström 1968, Suoranta and Karmano 1974).

The experiments reported in papers III and V were performed with a combination of angiographic and histologic

methods. With this technique it was possible to study the tissue reactions and the repair processes more in detail. It has been shown, however, that it might be difficult to distinguish between vital and nonvital tissue by routine histologic techniques (Mejare, Hasselgren and Hammarström 1975, Rölling, Hasselgren and Tronstad 1976, Hasselgren, Larsson and Rundquist 1977), and consequently, supplementary work using enzyme histochemical methods for demonstration of oxido-reductase activity was performed for this purpose (II).

In the present investigations care was taken to arrange as favourable conditions as possible for pulpal and periodontal repair. Thus the experiments on immature teeth (I, II,III) were performed when the foramina were wide open (Öhman 1965) and about three fourths of the roots were mineralized (Andreasen and Hjørting-Hansen 1966a), which enhanced the possibilities for pulpal repair and yet minimized the risk of exfoliation postoperatively. In mature teeth (IV,V) a method comprising apicoectomy with removal of about one fourth of the length of the root was used, and the root length was then on a par with the immature teeth. The area of the pulp exposed to the periapical tissue was thus markedly enlarged which should improve the revascularization capacity of the pulp. In order to minimize the operative trauma surgical preparation of the sockets was not performed (Löe and Waerhaugh 1961, Deeb et al. 1965, Sherman 1968, Bolton 1974). Moreover, efforts were made to avoid infection. So, for example, penicillin was given pre- and postoperatively. Further, the teeth were stored in sterile isotonic saline solution during the extraoral time, that did not exceed 10 minutes, to reduce the risk of ankylosis (Andreasen and Hjørting-Hansen 1966a, Anderson et al. 1968a, Andreasen et al. 1970, Çvek et al. 1974, Andreasen et al. 1978).

The precautions appeared to have been efficient since severe complications did not occur.

A majority of the replanted and autotransplanted immature teeth and a majority of the mature teeth with apicoectomized roots showed pulpal revascularization (I,IV). However, large areas of the pulp had apparently no vascular supply for days and even weeks after the operation. The revascularization rate was slower, as a rule, in the mature apicoectomized teeth than in the immature teeth.

It has been stated previously that the blood supply of transplants may be restored either by uniting vessels of the graft with vessels from the recipient bed (Peer 1959, Östrup and Fredricksen 1974), by spontaneous anastomoses between vessels from the graft and vessels from the recipient bed (Clemmesen 1967), by ingrowth of vessels from the host tissue into the graft (Clemmesen 1967, Alm and Strömberg 1974), or by combinations of these possibilities (Clemmesen 1967, Haller and Billingham 1967, Smahel and Ganzoni 1970). In tooth grafts revascularization primarily seems to occur by ingrowth of newly formed vessels. It has been found that blood vessels have a capacity to advance 0.1 - 0.6 mm per day (Anderson and Kissane 1977). Since revascularization was observed in a few of the immature teeth in the whole root pulp and even areas of the coronal pulp as early as 4 days postoperatively, formation of anastomoses was possible between vessels from the recipient bed and the pre-existing main vessels of the pulp. A prerequisite for spontaneous anastomoses during the first postoperative days is a close contact between the graft and the recipient bed (Smith, 1950), which might explain the finding that none of the apicoectomized teeth showed the signs of rapid revascularization.

The histochemical demonstrations of oxido-reductase activities (II) showed that there might be a possibility for survival of the original pulp tissue if revascularization should occur during 3-4 days after the

operation. Staining for enzyme activity was shown in the whole pulp during this period of time. Evidence of survival of the original pulp tissue rather than repair was noted in a few of the immature teeth (III). In these teeth a normal pulp tissue with an intact odontoblastic layer was observed, and obliteration of the root canal had not occurred, which is favourable (Andreasen 1972).

In the present material, however, a majority of the immature teeth (II,III) and all mature apicoectomized teeth (V), first showed pulpal necrosis. Then repair occurred with a well-vascularized, cell-rich connective tissue that reached the pulp horn after approximately 30 days in the immature teeth (III) and between 30 and 180 days in the mature teeth (V). Later this tissue was markedly reduced in the number of cells and blood vessels (III). Changes of this nature has also been noted in other parts of the body subjected to ischemic necrosis, for example, in the heart after coronary infarction (Anderson and Scotti 1972).

In most of the immature teeth (III) and all mature teeth with apicoectomized roots (V) a tissue resembling bone or cementum had been laid down on the root canal walls postoperatively. A similar hard tissue had also formed in some teeth in the central areas of the pulp. Such reactions are known from previous studies on replanted and autotransplanted immature teeth and may lead to obliteration of the root canal (Agnew and Fong 1956, Nordenram 1963, Öhman 1965, Monsour 1971). Extraosseous formation of bone has been found occasionally in scar tissue also in other regions of the body (Walter, Hamilton and Israel 1974, Anderson and Kissane 1977) and it has been proposed that fibroblasts might be capable of producing hard tissue matrix or that they undergo metaplasia to hard tissue-producing cells (Walter et al. 1974). Another

explanation of this phenomenon is that hard tissue-producing cells might be derived from primitive stem cells in the scar (Walter et al. 1974). Concerning tooth grafts, with an ingrown tissue from the apical region, the latter explantation appears to be the most likely.

During the period of pulpal repair large accumulations of extravasated erythrocytes were seen in the border zone between vascularized and nonvascularized tissue, but only a slight inflammatory reaction was noted (III,V). It has previously been shown that sterile necrotic tissue gives rise to only a mild inflammatory reaction in the adjacent tissue (Torneck 1967, Makkes, Toden van Velsen and Wesselink 1978). The results in the present studies (III,V) coincide with these previous findings.

The pulp of the mature nonapicoectomized replanted and autotransplanted teeth necrotized, and repair did not occur (V). This finding corresponded well with the results of previous experimental studies on adult dogs (Knight et al. 1964, Kosnevish 1974) and monkeys (Barbakow et al. 1977, Nasjleti et al. 1978), and also with findings in mature autotransplanted human teeth (Hasselgren et al. 1977).

It has been suggested that necrotic pulp tissue may give rise to external root resorption of the inflammatory type (Andreasen 1972). External root resorption affected all replanted and autotransplanted teeth in the present studies (III,V), but repair of the resorption cavities usually occurred simultaneously with the repair of the pulp (III,V). It was interesting to note that also some of the mature nonapicoectomized teeth showed repair of the resorption cavities, although pulpal repair had not occurred (V).

The results of the present studies showed that the replanted and autotransplanted immature teeth and mature

teeth with apicoectomized roots first became necrotic and then underwent repair (I,II,III). A few immature teeth showed evidence of pulpal survival (III). The mature non-apicoectomized teeth necrotized and repair did not occur (IV,V). Thus, the repair capacity of the pulp of mature teeth was markedly improved if apicoectomy was carried out at the time of the operation (IV,V). Before the apicoectomy technique is applied to the clinic, it should be borne in mind, however, that this study was performed on dogs, and that there might be differences between species as regards the repair capacity of the pulp. Further, possible clinical implications of pulpal obliteration should be considered and weighed against the disadvantages of endodontic treatment.

CONCLUSIONS

In the present investigations on dog teeth, the pulp usually necrotized after replantation or autotransplantation.

In immature teeth, pulpal repair occurred by ingrowth of a well-vascularized, cell-rich connective tissue that reached the pulp horn after approximately 30 days.

Mature teeth, that were replanted or autotransplanted without apicoectomy did not show pulpal repair.

The revascularization capacity of the pulp of mature teeth was markedly improved if apicoectomy was performed during the operation.

The repair process of the pulp proceeded considerably slower in the mature apicoectomized teeth than in the immature teeth, but otherwise in a similar way.

The ingrown soft tissue was gradually partly replaced by hard tissue both in immature teeth and in mature teeth with apicoectomized roots.

The pulp appeared to remain vital during the 3-4 first postoperative days, and a restoration of the vascular system during this period should make a tissue survival possible.

Some immature teeth exhibited a normal pulp morphology. It is assumed that the original pulp tissue in these teeth had survived after the operation.

All replanted and autotransplanted teeth were affected by external root resorption.

Repair of the resorption cavities usually occurred simultaneously with the repair of the pulp.

Repair of external resorption cavities was also noted in teeth with necrotic pulp tissue, indicating that there are also other factors operating in the resorption process.

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REFERENCES

Agnew, R.G., and Fong, C.C.: Histologic studies on experimental transplantation of teeth. Oral Surg. 1956:9:18-39.

Alm, A., and Strömberg, B.: Transposed medial third of patellar ligament in reconstruction of the anterior cruciate ligament. A surgical and morphologic study in dogs. Acta Chir. Scand. 1974:445:37-49.

Anderson, A.W., Sharav, Y., and Massler, M.: Periodontal reattachment after tooth replantation. Periodontics 1968:6:161-167.

Anderson, W.A.D., and Scotti, T.M.: Synopsis of Pathology. The C.V. Mosby Co., St. Louis 1972:347-351.

Anderson, W.A.D., and Kissane, J.M.: Pathology. The C.V. Mosby Co., St. Louis 1977:pp. 77, 85.

Andreasen, J.O., and Hjørting-Hansen, E.: Replantation of teeth. I. Radiographic and clinical study of 110 human teeth replanted after accidental loss. Acta Odontol. Scand. 1966a:24:263-286.

Andreasen, J.O., and Hjørting-Hansen, E.: Replantation of teeth. II. Histological study of 22 replanted anterior teeth in humans. Acta Odontol. Scand. 1966b:24: 287-306.

Andreasen, J.O., Hjørting-Hansen, E., and Jølst, O.: A clinical and radiographic study of 76 autotransplanted third molars. Scand. J. Dent. Res. 1970:78:512-523.

Andreasen, J.O.: Treatment of fractured and avulsed teeth. J. Dent. Child. 1971:38:29-48.

Andreasen, J.O.: Traumatic Injuries of the Teeth. Munksgaards, Copenhagen, Denmark 1972:141-231.

Andreasen, J.O., Reinholdt, J., Riis, I., Dybdahl , R., Söder, P.-Ö., and Otteskog, P.: Periodontal and pulpal healing of monkey incisors preserved in tissue culture before replantation. Int. J. Oral Surg. 1978: 7:104-112.

Barbakow, F.H., Austin, J.C., and Cleaton-Jones, P.E.: Experimental replantation of root canal filled and untreated teeth in the vervet monkey. J. Endod. 1977: 3:89-93.

Barka, T., and Anderson, P.J.: Histochemistry, Theory, Practice and Bibliography, Harper and Row, New York, 1963.

Bell, W.H.: Revascularization and bone healing after anterior maxillary osteotomy: a study using adult rhesus monkeys. J. Oral Surg. 1969:27:249-255.

Bolton, R.: Autogenous transplantation and replantation of teeth: report of 60 treated patients. Br. J. Oral Surg. 1974:12:147-165.

Bowdler, H.: Examples of reimplantation of teeth in young subjects. Proc. Roy. Soc. Med. 1955:48:1013-1015.

Clemmesen, T.: Experimental studies on the healing of free skin autografts. Dan. Med. Bull. 1967:14:1-43.

Çvek, M.: Treatment of non-vital permanent incisors with calcium-hydroxide. II. Effects on external root resorption in luxated teeth compared with effect of root filling with guttapercha. A follow-up. Odont. revy 1973:24:343-354.

Çvek, M., Granath, L.-E., and Hollender, L.: Treatment of non-vital permanent incisors with calcium hydroxide. III. Variation of occurrence of ankylosis of reimplanted teeth with duration of extra-alveolar period and storage environment. Odont. revy 1974:25:43-56.

Deeb, E., Prietto, P.P., and McKenna, R.C.: Reimplantation of luxated teeth in humans. J. South. California D. A. 1965:33 194-206.

Flath, J.: Klinische beobachtungen bei der echten transplantation von retinierten zähnen. Dtsch. Stomat. 1963:13:561-574.

Haller, J.A., and Billingham, R.E.: Studies of the origin of the vasculature in free skin grafts. Ann. Surg. 1967:166:896-901.

Hasselgren, G., Larsson, Å., and Rundquist, L.: Pulpal status after autogenous transplantation of fully developed maxillary canines. Oral Surg. 1977:44:106-112.

Heithersay, G.S.: Replantation of avulsed teeth. A review. Aust. Dent. J. 1975:20:63-72.

Kallionemi, H., and Oksala, E.: Significance of an open apex or fracture of the root tip for the prognosis of vital maxillary canine autotransplantation. Proc. Finn. Dent. Soc. 1977:73:126-132.

Knight, M.K., Gans, B.J., and Calandra, J.C.: The effect of root canal therapy on replanted teeth of dogs. A gross, roentgenographic and histologic study. Oral Surg. 1964:18:227-242.

Kosnevish, M.Y.: The state of the pulp in autotransplantation of living teeth in an experiment with resection and without resection of the top of the root. Stomatol. (Mosc) 1974:53(2):1-4.

Lovius, B.B.J., Atherton, J.D., Wynne, T.H.M., and Finch, L.D.: Autogenous tooth transplantation: a clinical and histological investigation. Br. J. Orthod. 1972:1:27-33.

Löe, H., and Waerhaug, J.: Experimental replantation of teeth in dogs and monkeys. Arch. Oral Biol. 1961:3: 176-184.

Lundskog, J., Bränemark, P.-I., and Lindström, J.: Biomicroscopic evaluation of microangiographic methods. Adv. Microcirc. 1968:1:152-160.

Makkes, D.C., Toden van Velzen, S.K., and Wesselink, P.R.: The response of the living organism to dead and fixed dead tissue. J. Endod. 1978:4:17-21.

Mejare, I., Hasselgren, G., and Hammarström, L.E.: Effect of formaldehyde-containing drugs on human dental pulp evaluated by enzyme histochemical technique. Scand. J. Dent. Res. 1975:83:29-36.

Monsour, P.N.T.: Pulpal changes following the reimplantation of teeth in dogs. A histological study. Aust. Dent. J. 1971:16:227-231.

Moss, J.P.: Autogenous transplantation of maxillary canines. J. Oral Surg. 1968:26:775-783.

Moss, J.P.: The indications for the transplantation of maxillary canines in the light of 100 cases. Br. J. Oral Surg. 1975:12:268-274.

Nasjleti, C.E., Caffesse, R.G., and Castelli, W.A.: Replantation and autotransplantation of mature teeth without endodontics in monkeys. J. Dent. Res. 1978: 54:650-658.

Natiella, J.R., Armitage, J.E., and Greene, G.W.: The replantation and transplantation of teeth. Oral Surg. 1970:29:397-413.

Nordenram, Å.: Autotransplantation of teeth. A clinical and experimental investigation. Acta Odontol. Scand. 1963:21:suppl. 33.

Oksala, E.: Autotransplantation of vital maxillary canines. A clinical and radiographic study. Proc. Finn. Dent. Soc. 1974:70:suppl. 1.

Oksala, E., and Kallionemi, H.: A longitudinal clinical and radiographic study of autotransplantation of the maxillary canine. Proc. Finn. Dent. Soc. 1977:73:117-125.

Peer, L.A.: Transplantation of tissues. Williams and Wilkins Co., Baltimore 1959:473-543.

Rubin, P.: Microangiography. Facts and Artifacts. Radiol. Clin. North. Am. 1964:2:499-513.

Rölling, I., Hasselgren, G., and Tronstad, L.: Morphologic and enzyme histochemical observations on the pulp of human primary molars 3 to 5 years after formocresol treatment. Oral Surg. 1976:42:518-528.

Seligman, A.M., Karnovsky, M.G., Wasserkrug, H.L., and Hanker, J.S.: Nondroplet ultrastructural demonstration of cytochrome oxidase activity with a polymerizing osmiophilic reagent, diaminobenzidine (DAB). J. Cell Biol. 1968:38:1-14.

Sherman, P.: Intentional replantation of teeth in dogs and monkeys. J. Dent. Res. 1968:47:1066-1071.

Singh, K.K., and Dudani, I.C.: Autogenous transplantation of developing mandibular third molars. J. Indian Dent. Assoc. 1970:42:199-212.

Slagsvold, O., and Bjercke, B.: Autotransplantation of premolars with partly formed roots. A radiographic study of root growth. Am. J. Orthod. 1974:66:355-366.

Smahel, J., and Ganzoni, N.: Contribution to the origin of the vasculature in free skin autografts. Br. J. Plast. Surg. 1970:23:322-325.

Smith, F.: Plastic and Reconstructive Surgery, W.B. Saunders Co., Philadelphia and London 1950, p. 26.

Suoranta, H., and Kormano, M.: Fixation-induced changes in the microangiographic picture of the blood vessels. Invest. Radiol. 1974:9:408-411.

Thonner, K.-E., and Meijer, M.: Autotransplantation or impacted upper canines. A clinical and histological investigation. Odont. T. 1969:77:113-119.

Torneck, C.D.: Reaction of rat connective tissue to polyethylene tube implants. Oral surg. 1967:24:647-683.

Walsh, J.S., Kafrawy, A., and Roche, R.R.: The effect of apical modification on the vitality of replanted permanent monkey teeth. J. Dent. Child. 1978:45:146-150.

Walter, J.B., Hamilton, M.C., and Israel, M.S.: Principles of Pathology for Dental Students. 3 ed. Churchill Livingstone, Edinburgh, London and New York 1974;p.238.

Widman, L.: Om transplantation av retinerade hörntänder. Sven. Tandlak. Tidskr. 1915:8:131-160.

Öhman, A.: Healing and sensitivity to pain in young replanted human teeth. An experimental, clinical and histological study. Odont. T. 1965:73:166-227.

Östrup, L.T., and Fredrickson, J.M.: Distant transfer of free living bone graft by microvascular anastomoses. Plast. Reconstr. Surg. 1974:54:274-285.

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A microangiographic study of vascular changes in replanted and autotransplanted teeth of young dogs

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The purpose of the present investigation was to study the revascularization process of the pulp of replanted and autotransplanted teeth with incomplete root development in dogs. A barium-sulfate injection method combined with contact microradiography was used. Ingrowth of apparently new vessels was seen during the first postoperative days. After 10 days, visible vessels were seen in the apical half of the pulp, and after 30 days, in the whole pulp. Branches and apparently also anastomoses between pulpal vessels were seen after 10 days but were especially numerous after 30 days. One hundred eighty days postoperatively, only one of fifteen teeth was devoid of visible vessels, indicating pulpal necrosis. Thirteen of the remaining fourteen teeth exhibited visible vessels throughout the entire length of the pulp. It seems, therefore, that replanted and autotransplanted teeth with open apices have a high potential for repair. The revascularization of the pulp appeared to occur mainly by ingrowth of new vessels. In some instances, however, anastomoses seemed to form to pre-existing vessels in the pulp.

Numerous investigations have been performed to study the outcome of replantation and transplantation of teeth.[1-13] In clinical examinations the state of the pulp has been assessed by means of sensitivity tests and radiographs.[1, 11] In experimental studies, possible changes in the pulp and the periapical tissues have been evaluated mainly with histologic techniques.[4, 10, 13] Still, little is known about the vascular repair of tooth grafts. It was the aim of the present investigation, therefore, to study the revascularization process of the pulp of replanted and autotransplanted teeth by a barium-sulfate injection method combined with contact microradiography.

MATERIAL AND METHODS

The experimental material consisted of sixty-nine incisors and first premolars in nine dogs which were approximately 4 months old at the beginning of the experiment (Table I). All teeth had open apices (Figs. 1 to 4). As controls, thirty-six intact teeth, one from each

Table I. Distribution of experimental material

	No. of teeth	*First incisor*	*Second incisor*	*Third incisor*	*First premolar*
Maxilla					
Replanted teeth	17	3	1	9	4
Transplanted teeth	18	8	7		3
Mandible					
Replanted teeth	16	7	5	4	
Transplanted teeth	18	2	3	5	8
Total	69	20	16	18	15

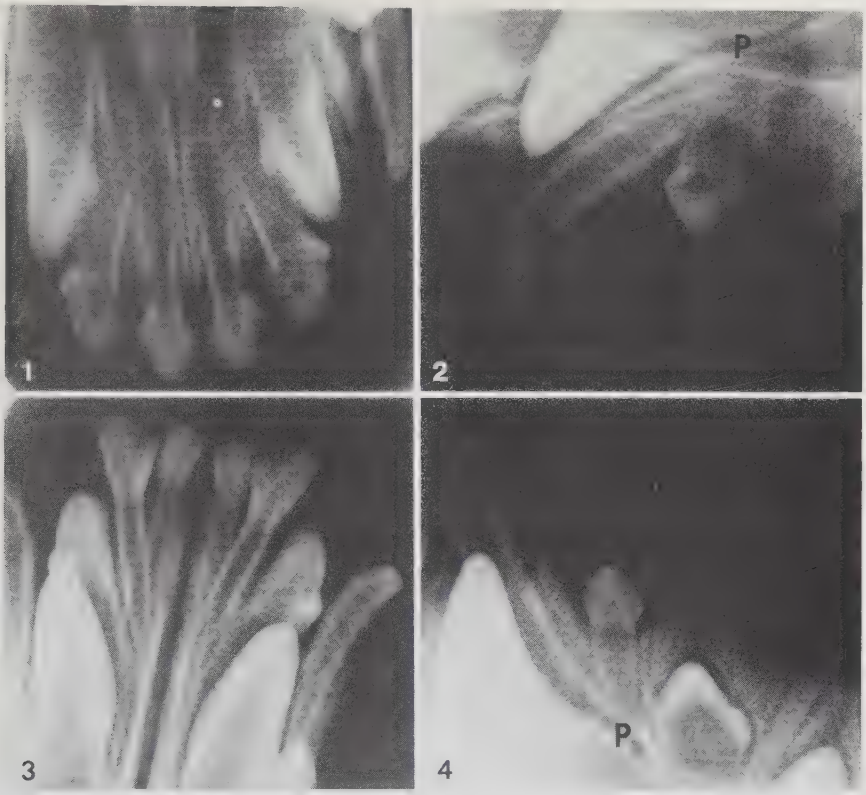
quadrant of the jaws, were used. The dogs were anesthetized by intravenous administration of a barbiturate solution (Pentothal sodium*). The teeth were extracted and either replanted or transplanted to the socket of the contralateral tooth. Surgical preparation of the sockets was not performed. Extraoral time did not exceed 10 minutes. The teeth were stored during this time in sterile isotonic saline solution at room temperature.

Intramuscular injections of penicillin† were given from 1 day before until 6 days after the operation (300,000 I.U. per day). Fixation of the teeth was not used. The dogs were put on a diet of soft food for 2 weeks or until the time of sacrifice. The observation periods chosen were 1, 4, 10, 30, and 180 days. The distribution of the experimental material is seen in Table II.

At the end of the observation periods, the animals were anesthetized by intravenous administration of a barbiturate solution.* Subcutaneous injections of heparin,† 500 I.U. per kilogram of body weight, were given. The left and right common carotid arteries were exposed and cannulated. The vessels were perfused with a warm (37° C.) barium-sulfate injection medium consisting of Micropaque‡ (60 percent) and isotonic saline solution (40 percent). The injection was performed by means of a pump with the use of a pressure slightly above the systolic blood pressure (170 mm. Hg). The jugular veins were cut. When the blood vessels under the tongue were filled with Micropaque and there was a flow of contrast medium from the jugular veins, the dogs were injected with a warm (37° C.) solution of Micropaque (60 percent) and 4 percent neutral-buffered formaldehyde (pH 7.4) (40 percent) until muscle fasciculations were noted.

The jaws were then excised and fixed in 4 percent neutral-buffered formaldehyde (pH 7.4). After demineralization in 5 percent nitric acid, longitudinal slices about 1 mm. thick were cut from the middle portion of the teeth with a scalpel in an axiobuccolingual direction. The slices were dehydrated and embedded in paraffin. Contact microradiographs were produced with a Philips diffraction generator (Pw 1010/25) and diffraction tube (25293/52) on Kodak spectroscopic plates 649-0 or Kodak high-resolution plates. An object holder and camera constructed by Löfgren and co-workers[14] were used. Copper

*Abbott, S.A., Brussels, Belgium.
†Suspenin Transin, Kabi, Stockholm, Sweden.
*Mebumal Vet, ACO, Solna, Sweden.
†Vitrum, Stockholm, Sweden.
‡Nicholas, Slough, Bucks, England.



Figs. 1 to 4. Radiographs of maxillary incisors (Fig. 1), maxillary first premolar (**P**) (Fig. 2), mandibular incisors (Fig. 3), and mandibular first premolar (**P**) (Fig. 4) at the time of operation. The root development varies somewhat, but all teeth have open apices.

Table II. Distribution of experimental material according to observation periods

	No. of teeth	*1 day*	*4 days*	*10 days*	*30 days*	*180 days*
Replanted teeth	33	3	9	5	10	6
Transplanted teeth	36	6	7	7	7	9
Total	69	9	16	12	17	15

radiation at 35 kv. and 20 Ma. was employed. The target-film distance was 300 mm., and the average exposure time was 5 minutes. The plates were developed in Kodak D-19 under constant agitation for about 4 minutes.

RESULTS

Control teeth

The vascular pattern of the pulp was well demonstrated with the present technique. The larger blood vessels were centrally located and ran mainly parallel with each other in a longitudinal direction from the apical foramina to the coronal pulp or vice versa (Fig. 5).

Table III. Area of pulp containing vessels filled with contrast medium

Observation periods (days)	*No. of teeth*	*Area of pulp with visible vessels*			
		None to one fourth	*One fourth to two fourths*	*Two fourths to three fourths*	*Three fourths to four fourths*
1	9	9			
4	16	12	3		1
10	12	3	8	1	
30	17			2	15
180	15	1		1	13

The main arteries and veins were surrounded by a number of intermediate-sized vessels from which numerous small branches passed radially to terminate in a subdentinal capillary plexus (Figs. 5 and 6). Smaller vessels and branches were especially numerous in the coronal pulp (Fig. 5).

Experimental teeth

No difference was noted between the reaction of the replanted teeth and that of the autotransplanted teeth. Nor was there any difference in the reaction of maxillary and mandibular teeth from different regions of the jaws.

One day. No visible vessels were seen in the pulp at this observation time (Fig. 7, Table III).

Four days. A few slender Micropaque-filled vessels were seen in the most apical area of the pulp (Table III). The vessels were separated from each other and, for the most part, had a direction parallel with the long axis of the tooth (Fig. 8). In three teeth some larger vessels were seen extending about half the length of the pulp. In one instance a few main vessels with tiny branches ran throughout almost the entire pulp (Fig. 9).

Ten days. A great number of Micropaque-filled vessels were noticed in the pulp of all teeth at this observation time. The vessels were usually separated from each other and ran in a direction parallel with the dentinal walls, but numerous branches were seen (Figs. 10 and 11). The area which was vascularized varied in size but did not exceed the apical three quarters of the pulp (Table III).

Thirty days. In two teeth only the root pulp exhibited visible vessels, whereas in fifteen teeth the whole pulp contained vessels filled with contrast medium (Table III). The number of visible vessels had increased greatly between 10 and 30 days, and in addition to the longitudinally coursing vessels, a network of branches and apparently also anastomoses between vessels were seen (Figs. 12 and 13). A subdentinal capillary network was not visible in five teeth (Fig. 14). In the remainder of the teeth, such a network was seen more or less distinctly in different areas of the root portion of the pulp (Figs. 12, 13 and 15).

One hundred eighty days. In thirteen of fifteen teeth the entire pulp contained vessels filled with contrast medium (Fig. 16, Table III). However, a subdentinal capillary network similar to that observed in the control teeth was seen in only one of these teeth (Fig. 17). One tooth contained vessels only in the apical three fourths of the pulp, whereas the pulp of one tooth was completely devoid of contrast medium (Table III).

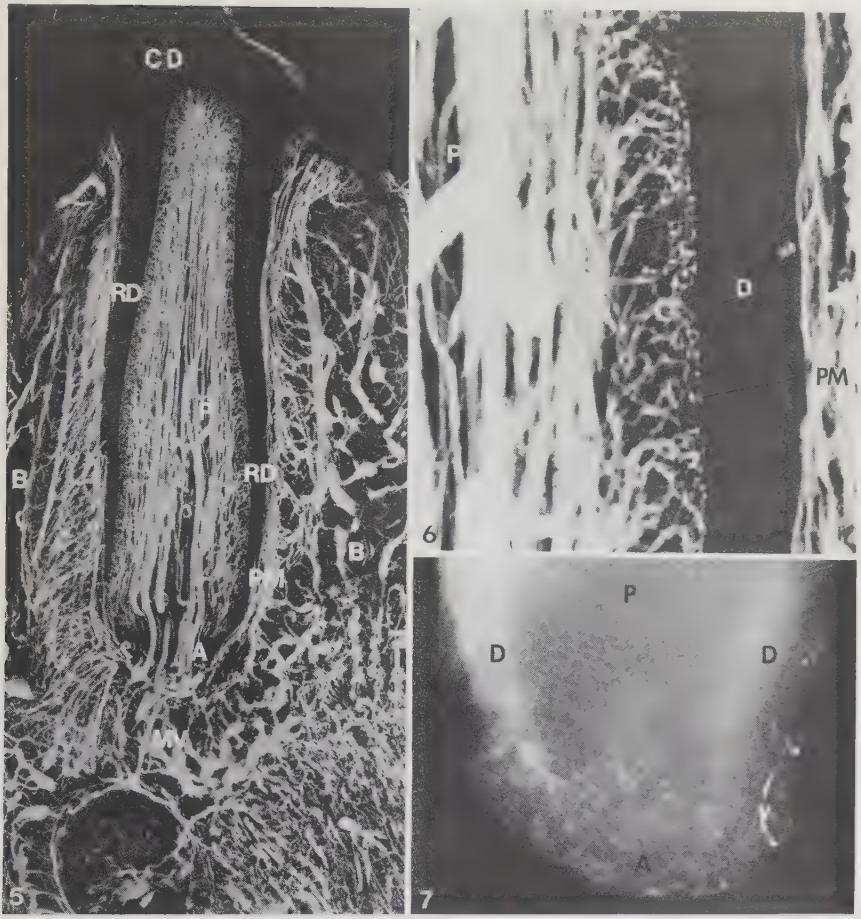


Fig. 5. Microangiograph of control tooth (mandibular third premolar). A main vessel (**MV**) is dividing into branches entering the periodontal membrane (**PM**) and the pulp (**P**). A bundle of central pulpal vessels ascends the radicular and coronal portion of the pulp, giving off branches in great number, especially in the coronal pulp. The main arteries and veins are surrounded by intermediate-sized vessels, from which numerous smaller branches pass radially to terminate in a subdentinal capillary plexus. **A,** Apical region. **B,** Bone. **RD,** Root dentin. **CD,** Coronal dentin. (Magnification, ×15.)

Fig. 6. Detail from Fig. 5. Note the rich vascularization of subdentinal area. **D,** Dentin. **P,** Pulp (marked at the same place as **P** in Fig. 5). **PM,** Periodontal membrane. (Magnification, ×100.)

Fig. 7. Microangiograph of autotransplanted second maxillary incisor, 1 day postoperatively. There are no visible vessels in the pulp (**P**). The damage to the periodontium is marked at this observation time, and the teeth and surrounding tissues are, as a rule, disunited during sectioning. **A,** Apical region. **D,** Dentin.

DISCUSSION

Previous research has shown that many factors may influence the healing process after replantation or autotransplantation of teeth.[4, 6, 10, 15, 16] In the present study, we took care to create optimal conditions for vascular repair. Thus, we used teeth with open apices[15] but with three quarters of their roots mineralized.[16] Furthermore, in order to minimize

Fig. 8. Microangiograph of replanted third mandibular incisor, 4 days postoperatively. A few tiny vessels are seen in the apical region **(A)** of the pulp **(P).** (Magnification, ×10.)

Fig. 9. Microangiograph of replanted first maxillary premolar, 4 days postoperatively. Some slender vessels are visible in the apical region of the pulp **(A),** but, in addition, a few larger vessels are seen running all the way to the coronal pulp **(C).** Some branches are noted. **B,** Bone. **D,** Dentin. (Magnification, ×15.)

operative trauma,[7] surgical preparation of the sockets was not performed, and the teeth were stored in saline solution during the extraoral period, which was kept as short as possible.[16]

It has been shown that injection methods are suitable in revascularization research.[17–23] In the present study a microangiographic technique was used. It permits study of the vasculature in greater depth than is possible with routine histologic procedure. Of special importance is the fact that only vessels involved in the circulation are registered. It should be borne in mind, however, that an exact picture of the actual intravital microvascular anatomy is not obtained.[24, 25]

The major artifacts of microangiographic techniques arise from overfilling or poor filling of the vessels with contrast medium.[26] However, when proper precautions are

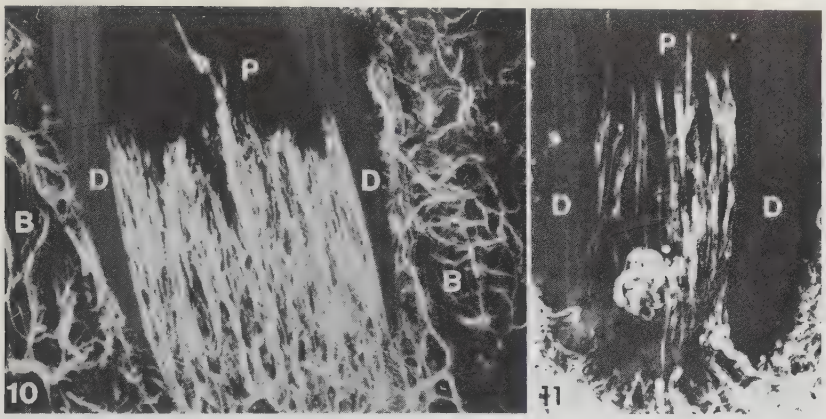


Fig. 10. Microangiograph of autotransplanted first maxillary incisor, 10 days postoperatively. A great number of Micropaque-filled vessels with numerous branches are seen extending about half the length of the pulp **(P). B,** Bone. **D,** Dentin. (Magnification, ×15.)

Fig. 11. Microangiograph of autotransplanted first maxillary premolar, 10 days postoperatively. Blood vessels are seen entering the apical foramina and running parallel with the long axis of the tooth for about one quarter of the length of the pulp **(P).** A coil of blood vessels is seen in the most apical region. **D,** Dentin. (Magnification, ×50.)

taken, these errors are small. Overfilling, which is due mainly to a high injection pressure or a closed injection system, was not a major problem in the present investigation. The contrast medium was injected with a constant pressure slightly above the systolic blood pressure, and the jugular veins were cut during the injection. Only occasionally were ruptured vessels seen, and these were situated, for the most part, in the tissues surrounding the teeth.

Poor filling of the vessels is mainly due to low injection pressure, large particle size of the contrast medium, leakage of medium, vascular spasm, or blood clotting. The contrast medium used in the present study (Micropaque) has a particle size smaller than the capillary diameter.[26] In order to reduce leakage, the injection medium is often mixed with gelatin.[26] However, when blood is replaced by a gelatinous contrast medium, the animal will not be in a physiologic state for very long. Also, the medium quickly solidifies when cooled. In a pilot study, the Micropaque-gelatin technique was compared to a technique that used a mixture of Micropaque and saline solution followed by Micropaque and 4 percent neutral-buffered formaldehyde.[17, 18] Better results were obtained with the latter method, which consequently was employed in the present investigation.

In the pilot study it was also found that, although the latter technique was used with a warm (37° C.) injection medium to prevent vascular spasm and heparin to prevent blood clotting, poor filling occurred if the jugular veins were cut at the beginning of the injection. This difficulty was overcome if the veins were not cut until the arterial capillaries under the tongue were almost completely filled with contrast medium. The experimental conditions were further controlled by examination of one intact tooth from each quadrant of the jaws, and all control teeth exhibited a vascular pattern similar to that described by Saunders[27, 28] and Kramer[29] in the pulp of human and monkey teeth.

In the literature on revascularization of grafts, tissues, or organs, it has been stated that

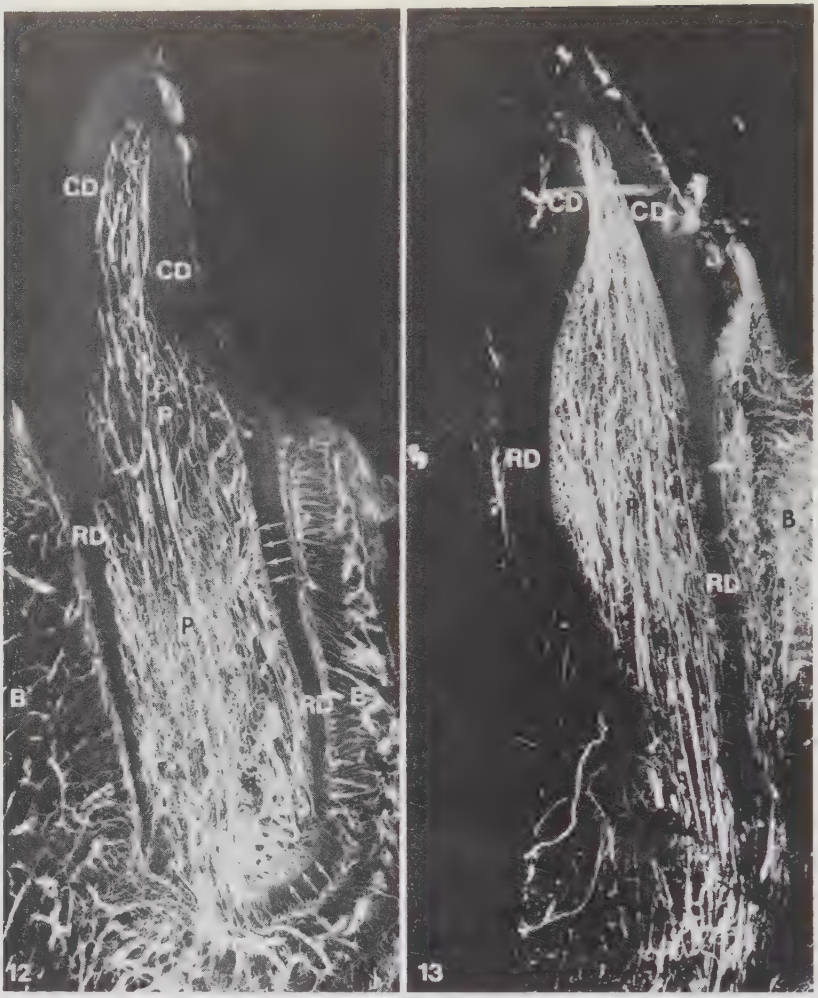


Fig. 12. Microangiograph of replanted third maxillary incisor, 30 days postoperatively. Numerous vessels run throughout the entire length of the pulp **(P).** Branches and apparently also anastomoses between vessels are seen. In some areas of the root pulp a subdentinal capillary plexus is seen **(arrows). B,** Bone. **RD,** Root dentin. **CD,** Coronal dentin. (Magnification, ×15.)

Fig. 13. Microangiograph of autotransplanted third mandibular incisor, 30 days postoperatively. A subdentinal capillary plexus is seen in the radicular as well as in the coronal pulp. **B,** Bone. **RD,** Root dentin. **CD,** Coronal dentin. **P,** Pulp. (Magnification, ×15.)

the blood supply may be restored by uniting vessels of the graft with vessels of the recipient bed,[19, 30] by spontaneous anastomoses between vessels from the graft and vessels from the recipient area,[20] by ingrowth of vessels from the host tissue into the graft,[20, 21] or by combinations of these events.[20, 22, 23]

Apparently, revascularization of the pulp of tooth grafts primarily occurs by ingrowth of newly formed blood vessels. In the present study, slender, longitudinally oriented vessels invaded the apical region of the pulp during the first days postoperatively. In some

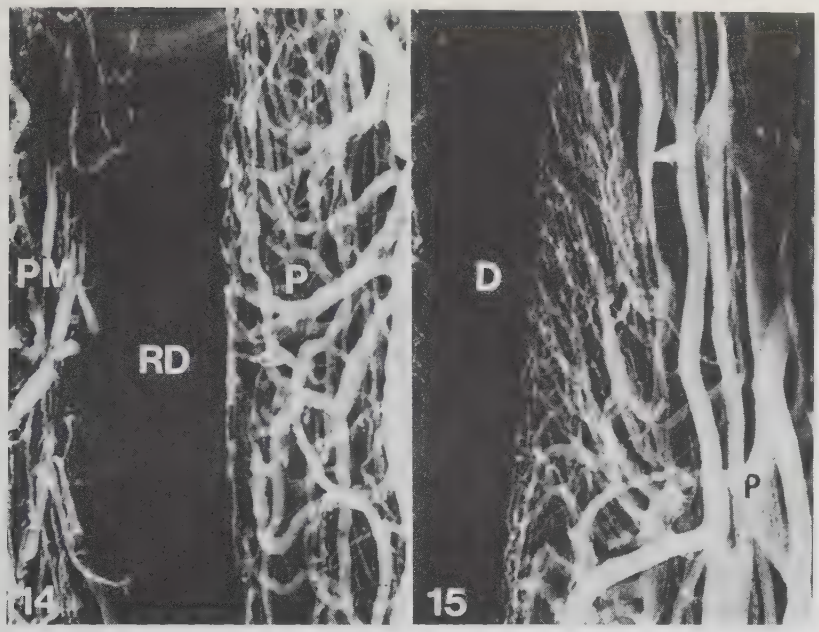


Fig. 14. Microangiograph of replanted third maxillary incisor, 30 days postoperatively. The typical subdentinal capillary plexus is missing. A number of vessels of intermediate size are seen adjacent to the root dentin **(RD). P,** Pulp. **PM,** Periodontal membrane. (Magnification, ×60.)

Fig. 15. Microradiograph of replanted second mandibular incisor, 30 days postoperatively. A subdentinal capillary plexus is seen, although it is not so distinct as in the control teeth. **D,** Dentin. **P,** Pulp. (Magnification, ×60.)

instances, however, anastomoses seemed to form to pre-existing, mostly larger vessels in the pulp (Fig. 9). After 10 days approximately half of the pulp was revascularized, and 30 days postoperatively numerous smaller and larger vessels with a vast number of branches and ramifications were observed in the whole pulp in fifteen of seventeen teeth. The subdentinal network which was a striking observation in all control teeth was by far not fully restored after 30 days. One hundred eighty days postoperatively, only one out of fifteen teeth studied was devoid of visible vessels, thus indicating pulpal necrosis. In the other fourteen teeth, thirteen exhibited visible vessels throughout the entire length of the pulp. It seemed, however, as though the number of visible vessels had decreased somewhat between 30 and 180 days, especially in the coronal pulp, and a subdentinal plexus was not observed in any of the teeth but one. This observation may be due to experimental variables, but other explanations are conceivable, for instance, that a mineralization of areas of the pulp tissue has occurred.[4, 10, 31]

In the present study, large areas of the pulp of the tooth grafts had no vascular supply for days and weeks postoperatively, and the slow rate of revascularization of the pulp was somewhat surprising. Full-thickness skin grafts, for instance, are fully revascularized after 6 days.[20] However, the pulp is apparently not unique in this respect. It is known that revascularization of other tissues in the organism may take weeks and even months to be completed. The vascular zone of a transposed patellar ligament, for example, is invaded

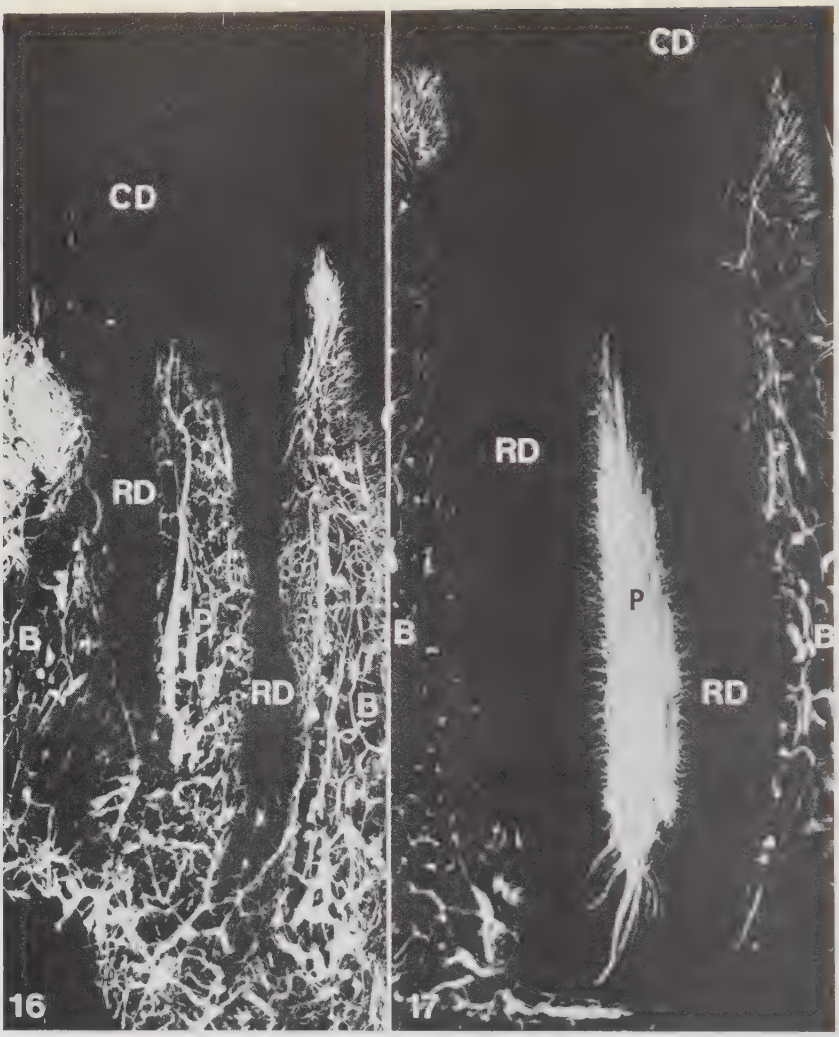


Fig. 16. Microradiograph of replanted first maxillary premolar, 180 days postoperatively. Blood vessels are seen throughout the entire length of the pulp **(P).** No subdentinal plexus is visible. **B,** Bone. **RD,** Root dentin. **CD,** Coronal dentin. (Magnification, ×15.)

Fig. 17. Microangiograph of autotransplanted second maxillary incisor, 180 days postoperatively. Blood vessels are seen throughout the entire length of the pulp **(P),** and a subdentinal capillary plexus is clearly visible. **B,** Bone. **RD,** Root dentin. **CD,** Coronal dentin.

by newly formed, mainly longitudinally oriented vessels during the first 8 postoperative weeks.[21] These findings are remarkable similar to the findings of the present investigation, and Alm and Strömberg[21] have stated that a transposed patellar ligament can survive although parts of it are without a blood supply for weeks. Apparently, this is the case with the pulp of not fully developed tooth grafts as well.

This observation raises many interesting questions with regard to nutrition, metabolism, and tissue reactions in the pulp. Apparently, there is a transport of fluids between the

recipient site and the graft before the blood supply is restored.[20, 31] However, this process is not fully understood, so that a study of the metabolism of the pulp of replanted and transplanted teeth during the revascularization period should be of interest.

REFERENCES

1. Moss, J. P.: Autogenous Transplantation of Maxillary Canines, J. Oral Surg. **26:** 775-783, 1968.
2. Nordenram, Å.: Autotransplantation of Teeth, Br. J. Oral Surg. **7:** 188-195, 1969-1970.
3. Andreasen, J. O., Hjørting-Hansen, E., and Jølst, O.: A Clinical and Radiographic Study of 76 Autotransplanted Third Molars, Scand. J. Dent. Res. **78:** 512-523, 1970.
4. Natiella, J. R., Armitage, J. E., and Greene, G. W.: The Replantation and Transplantation of Teeth, ORAL SURG. **29:** 397-419, 1970.
5. Slagsvold, O.: Autotransplantation of Premolars in Cases of Missing Anterior Teeth, Trans. Eur. Orthod. Soc. 46th Congr., pp. 473-485, 1970.
6. Singh, K. K., and Dudani, I. C.: Autogenous Transplantation of Developing Mandibular Third Molars, J. Indian Dent. Assoc. **42:** 199-212, 1970.
7. Bolton, R.: Autogenous Transplantation and Replantation of Teeth: Report on 60 Treated Patients, Br. J. Oral Surg. **12:** 147-165, 1974.
8. Oksala, E.: Autotransplantation of Vital Maxillary Canines. A Clinical and Radiographic Study, Proc. Finn. Dent. Soc. **70:** Suppl. 1, 1974.
9. Slagsvold, O., and Bjercke, B.: Autotransplantation of Premolars With Partly Formed Roots, Am. J. Orthod. **66:** 355-366, 1974.
10. Heithersay, G. S.: Replantation of Avulsed Teeth. A Review, Aust. Dent. J. **20:** 63-72, 1975.
11. Moss, J. P.: The Indications for the Transplantation of Maxillary Canines in the Light of 100 Cases, Br. J. Oral Surg. **12:** 268-274, 1975.
12. Zemchenkov, V. N.: The Effect of Position of Transplanted Dental Rudiments in Respect to the Mandibular Vaso-Nervous Bundle on Their Take and Development, Stomatologiia (Mosk.) **53:** 4-7, 1974.
13. Barbakow, F. H., and Cleaton-Jones, P. E.: Experimental Replantation of Root-canal-filled and Untreated Teeth in the Vervet Monkey, J. Endod. **3:** 89-93, 1977.
14. Löfgren, L.-G., Omnell, K.-Å., and Svensson, A.: Object Holders and Cameras for Contact Microradiography, Acta Odontol. Scand. **24:** 563-578, 1966.
15. Öhman, A.: Healing and Sensitivity to Pain in Young Replanted Human Teeth. An Experimental, Clinical and Histological Study, Odontol. Tidskr. **73:** 166-227, 1965.
16. Andreasen, J. O., and Hjørting-Hansen, E.: Replantation of Teeth. I. Radiographic and Clinical Study of 110 Human Teeth Replanted After Accidental Loss, Acta Odontol. Scand. **24:** 263-286, 1966.
17. Bell, W. H.: Revascularization and Bone Healing After Anterior Maxillary Osteotomy: A Study Using Adult Rhesus Monkeys, J. Oral Surg. **27:** 249-255, 1969.
18. Bell, W. H., and Kennedy, J. W.: Biological Basis for Vertical Ramus Osteotomies—A study of Bone Healing and Revascularization in Adult Rhesus Monkeys, J. Oral Surg. **34:** 215-224, 1976.
19. Östrup, L. T., and Fredrickson, J. M.: Distant Transfer of a Free Living Bone Graft by Microvascular Anastomoses, Plast. Reconstr. Surg. **54:** 274-285, 1974.
20. Clemmesen, T.: Experimental Studies on the Healing of Free Skin Autografts, Dan. Med. Bull. **14:** 1-43, 1967.
21. Alm, A., and Strömberg, B.: Transposed Medial Third of Patellar Ligament in Reconstruction of the Anterior Cruciate Ligament. A Surgical and Morphologic Study in Dogs, Acta Chir. Scand. **445:** 37-49, 1974.
22. Haller, J. A., and Billingham, R. E.: Studies of the Origin of the Vasculature in Free Skin Grafts, Ann. Surg. **166:** 896-901, 1967.
23. Smahel, J., and Ganzoni, N.: Contribution to the Origin of the Vasculature in Free Skin Autografts, Br. J. Plast. Surg. **23:** 322-325, 1970.
24. Lundskog, J., Brånemark, P.-I., and Lindström, J.: Biomicroscopic Evaluation of Microangiographic Methods, Adv. Microcirc. **1:** 152-160, 1968.
25. Suoranta, H., and Kormano, M.: Fixation-Induced Changes in the Microangiographic Picture of the Blood Vessels, Invest. Radiol. **9:** 408-411, 1974.
26. Rubin, P.: Microangiography—Facts and Artifacts, Radiol. Clin. North Am. **2:** 499-513, 1964.
27. Saunders, R. L. de C. H.: Microradiographic Studies of Human Adult and Fetal Dental Pulp Vessels. *In* Cosslett, V. E. Engström, A., and Pattee, H. H., editors: X-ray Microscopy and Microradiography, New York, 1957, Academic Press, Inc., pp. 561-571.
28. Saunders, R. L. de C. H.: Microangiographic Studies of Periodontic and Dental Pulp Vessels in Monkey and Man, J. Can. Dent. Assoc. **33:** 245-252, 1967.

29. Kramer, I. R. H.: The Vascular Architecture of the Human Dental Pulp, Arch. Oral Biol. **2:** 177-189, 1960.
30. Peer, L. A.: Transplantation of Tissues, Baltimore, 1959, William & Wilkins Company, pp 473-543.
31. Nordenram, Å.: Autotransplantation of Teeth. A Clinical and Experimental Investigation, Acta Odontol. Scand. **21:** Suppl. 33, 1963.

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II.

OXIDO-REDUCTASE ACTIVITY IN THE PULP OF REPLANTED AND AUTOTRANSPLANTED TEETH OF YOUNG DOGS

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ABSTRACT

The purpose of the present investigation was to assess the vitality of the pulp of replanted and autotransplanted teeth with incomplete root formation by means of histochemical demonstration of oxido-reductase activity during a 180-day period.

Staining for enzyme activity was seen in the whole pulp during the first postoperative days. After 10 days staining was observed only in the foraminal area of the pulp and in blood vessels in apparent necrotic tissue. 30 days postoperatively staining was observed in the whole pulp. 180 days after replantation or autotransplantation six out of eight teeth exhibited enzyme activity in the entire pulp. Thus, it appeared that the pulp of the replanted and autotransplanted teeth with partly formed roots first underwent necrosis and that the dead tissue then was replaced by new tissue.

INTRODUCTION

Different opinions exist to which extent the original pulp tissue survives or undergoes necrosis after replantation or autotransplantation of teeth.[1-5] This may be due to the fact that routine histologic techniques have been used in most studies, and that it is hard to assess the vitality of the pulp tissue with such techniques, especially during the first postoperative days.

In a recent investigation in young dogs it was shown by means of a microangiographic technique that the pulp of replanted and autotransplanted teeth usually revascularized.[6] However, most of the pulp tissue was initially without vascular supply for days and even weeks until revascularization occurred.[6] The purpose of the present investigation was to assess by enzyme histochemical means the vitality of the pulp of replanted and autotransplanted

teeth with incompletely formed roots during and after the period of pulpal revascularization.

MATERIAL AND METHODS

The material comprised twenty-five single-rooted teeth (incisors and first premolars) from three dogs that were about four months old at the beginning of the experiment. The dogs were anesthetized by intravenous injection of Penthotal sodium® (Abbott S.A., Bruxelles, Belgium). After extraction thirteen teeth were replanted and twelve autotransplanted to the socket of the contralateral tooth. The extra-alveolar time did not exceed 10 min. and during this time the teeth were kept in sterile isotonic saline at room temperature. The dogs were given 30.000 I.U. penicillin (Suspenin, Transin®, Kabi, Stockholm, Sweden) per day intramuscularly from one day before until six days after the operation.

The teeth were not fixed and the dogs were given soft food for two weeks or until death. The observation periods were 1,2,4,10,30, and 180 days (Table 1). At the end of the observation periods the animals were anesthetized by an intravenous injection of a barbiturate solution (Mebumal Vet®, ACO, Solna, Sweden) and sacrificed by an intracardiac injection of the same solution.

The jaws were immediately excised and frozen in hexane cooled with solid CO_2 ($-75^{\circ}C$). Still frozen the jaws were radiographed and by means of a saw divided into pieces containing one to three teeth. These pieces were embedded in an aqueous solution of carboxymethylcellulose applied on a microtome stage. According to the method of Ullberg[7] the specimens were sectioned at $-18^{\circ}C$, and 10-20 µm thick longitudinal sections were taken in a bucco-lingual direction through the teeth. Scotch tape no. 688 (3M, St. Paul, USA) was used to stabilize the tissues during this procedure. The sections were freeze-dried for at

least two days and then brought to room temperature in an air-tight box in order to protect the tissues from condensation of moisture.

Sections attached to the tape were then incubated for histochemical demonstration of succinate dehydrogenase (E.C. 1.3.99.1), lactate dehydrogenase (E.C. 1.1.1.27), glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49), and cytochrome oxidase (E.C. 1.9.31).

The incubation for the three dehydrogenases were carried out at 37°C for 60 minutes according to the methods described by Barka and Anderson[8] and the incubations for cytochrome oxidase were performed according to Seligman et al.[9] at 37°C for 90 minutes. For the purpose of control some sections were kept in isotonic saline solution at 100°C for 15 minutes prior to incubation. Furthermore, sections from intact teeth were incubated together with the sections from the replanted and autotransplanted teeth. After incubation all sections were rinsed in distilled water and incubated in glycerin jelly. All chemicals were obtained from the Sigma Chemical Company, St. Louis, U.S.A.

RESULTS

Sections subjected to hot saline (100°C) showed no staining for enzyme activity after incubation. In all other sections staining for enzyme activity was seen in the tissues surrounding the teeth (e.g. bone, muscles, oral mucosa). The staining for oxido-reductase activity that was noted in the pulp tissue of replanted or autotransplanted teeth was always compared to this periradicular staining within the same section. Similar distribution patterns for the four enzymes were noted within the different teeth.

Intact teeth

Staining for the four oxido-reductases was found in all cells. In the pulp there was a markedly high activity in the odontoblasts.

Experimental teeth

Similar findings were made in replanted and autotransplanted teeth.

1 and 2 days postoperatively. Staining for enzyme activity was demonstrated in all cells of the pulp. The highest activity was noted in the odontoblasts (Fig. 1). In some of the teeth, the coronal part of the pulp showed a somewhat lower activity than the apical part.

4 days postoperatively. Enzyme activity was demonstrated in the entire pulp tissue. However, different levels of activity were observed in different regions of the pulp (Fig. 2). There was a rather high activity in the foraminal area and a weak activity in the coronal part. The border between the areas with different levels of activity was formed like an apically pointing wedge (Figs. 2 and 3). Within each area the enzyme activity in the odontoblastic layer was higher than in the subjacent pulp tissue. Also, a high oxido-reductase activity was noted in the walls of some of the blood vessels entering the coronal part of the pulp (Fig. 3).

10 days postoperatively. Oxido-reductase activity was observed in the foraminal area of the pulp. Staining was also demonstrated in blood vessels reaching through approximately half the length of the pulp. A complete absence of enzyme activity was noted in the rest of the pulp (Fig. 4).

30 days postoperatively. A high enzyme activity was seen in the whole pulp cavity and the highest activity was noted in the coronal part of the pulp (Fig. 5). A zone, corresponding to the odontoblastic layer activity of

intact teeth was not observed.

180 days postoperatively. In six out of eight teeth oxido-reductase activity was demonstrated throughout the pulp (Fig. 6). In two instances (one replanted and one auto-transplanted tooth) only the root pulp showed enzyme activity (Fig. 7). Hard tissue was present in large areas of the pulp in seven teeth. Odontoblastic layer activity was not observed.

DISCUSSION

It has been found that it is not always possible to distinguish between vital and non-vital tissue by routine histologic techniques, and therefore histochemical demonstration of oxido-reductase activity has been used for this purpose.[10-12] In the present investigation the same methods were used to discriminate between vital and non-vital pulp tissue.

Enzyme activity was observed in all areas of the pulp 1 and 2 days after the operation, although sometimes the activity was reduced in the coronal regions. After 4 days a weak staining for enzyme activity was still observed throughout the entire pulp showing that metabolic activity was present in the tissue but also that the tissue now apparently was dying. However, there seems to be a possibility that the original pulp tissue may survive if revascularization should occur within 3-4 days after replantation or transplantation. In a microangiographic study, evidence of such a rapid revascularization was occasionally observed.[6]

After 10 days no oxido-reductase activity was seen in the pulp with the exception of the foraminal part of the tissue. An interesting finding at this observation time was the enzyme activity in blood vessels entering the necrotic

part of the pulp. As would be expected it appears that the repair process started with revascularization, either by ingrowth of new blood vessels penetrating the necrotic tissue or recanalization of old blood vessels.

30 days postoperatively there was a high enzyme activity in the tissue of the entire pulp cavity. This shows that the replacement of the necrotic tissue had made great progress between 10 and 30 days postoperatively. However, since the enzyme activity was especially high in the coronal area, it appears that the formation of the new tissue was not completed, at least not in the coronal part of the pulp cavity.

In most instances enzyme activity was observed in the whole pulp also 180 days postoperatively. However, two out of eight teeth exhibited vital tissue only in the root pulp. There seems to be two possible explanations of this finding. First, the ingrowth of new tissue might not have reached the most distant part of the pulp cavity in these teeth, or second, a newly formed tissue in the coronal pulp cavity might have necrotized for instance because of poor blood supply.

In conclusion, only the tissue of the foraminal part of the root canal remained vital after replantation or autotransplantation of dog teeth with incompletely formed roots. The rest of the pulp tissue of these teeth did not survive the operation, but necrotized, and was replaced by ingrowth of new tissue from the foraminal region of the root canal.

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Table 1. Distribution of experimental material

	No of teeth	Observation period (days) 1	2	4	10	30	180
Replanted teeth	13	2	1	2	3	1	4
Autotransplanted teeth	12	2	2	1	2	1	4
	25	4	3	3	5	2	8

LEGENDS

Fig. 1. Freeze-sectioned replanted third maxillary incisor, incubated for lactate dehydrogenase activity, 1 day postoperatively. Staining for enzyme activity is observed throughout the entire length of the pulp (P). High staining intensity is seen in the odontoblastic and subodontoblastic cell layers (arrows). The tooth has been extracted prior to freezening to facilitate sectioning. CD, coronal dentin. RD, root dentin. (Magnification, x 15.)

Fig. 2. Freeze-sectioned replanted third maxillary incisor, incubated for lactate dehydrogenase activity, 4 days postoperatively. Staining for enzyme activity is observed in the oral mucosa (OM), periodontal membrane (PM) and bone (B). Staining is also noted in the entire pulp (P), but a markedly higher activity is seen in the apical region than further coronally. A high activity is also noted in some blood vessels reaching from the apical region into the coronal part of the pulp. CD, coronal dentin. RD, root dentin. (Magnification, x 15.)

Fig. 3. Detail from Fig. 2. Apical region. Note that the staining for the highest enzyme activity in the apical part of the pulp (P) reaches somewhat more coronally near the dentin than centrally. PM, periodontal membrane. RD, root dentin. (Magnification, x 150.)

Fig. 4. Freeze-sectioned autotransplanted second incisor incubated for lactate dehydrogenase activity, 10 days postoperatively. Staining for enzyme activity is observed in the apical region (arrows) of the pulp (P) especially adjacent to and inside blood vessels. RD, root dentin. (Magnification, x 15.)

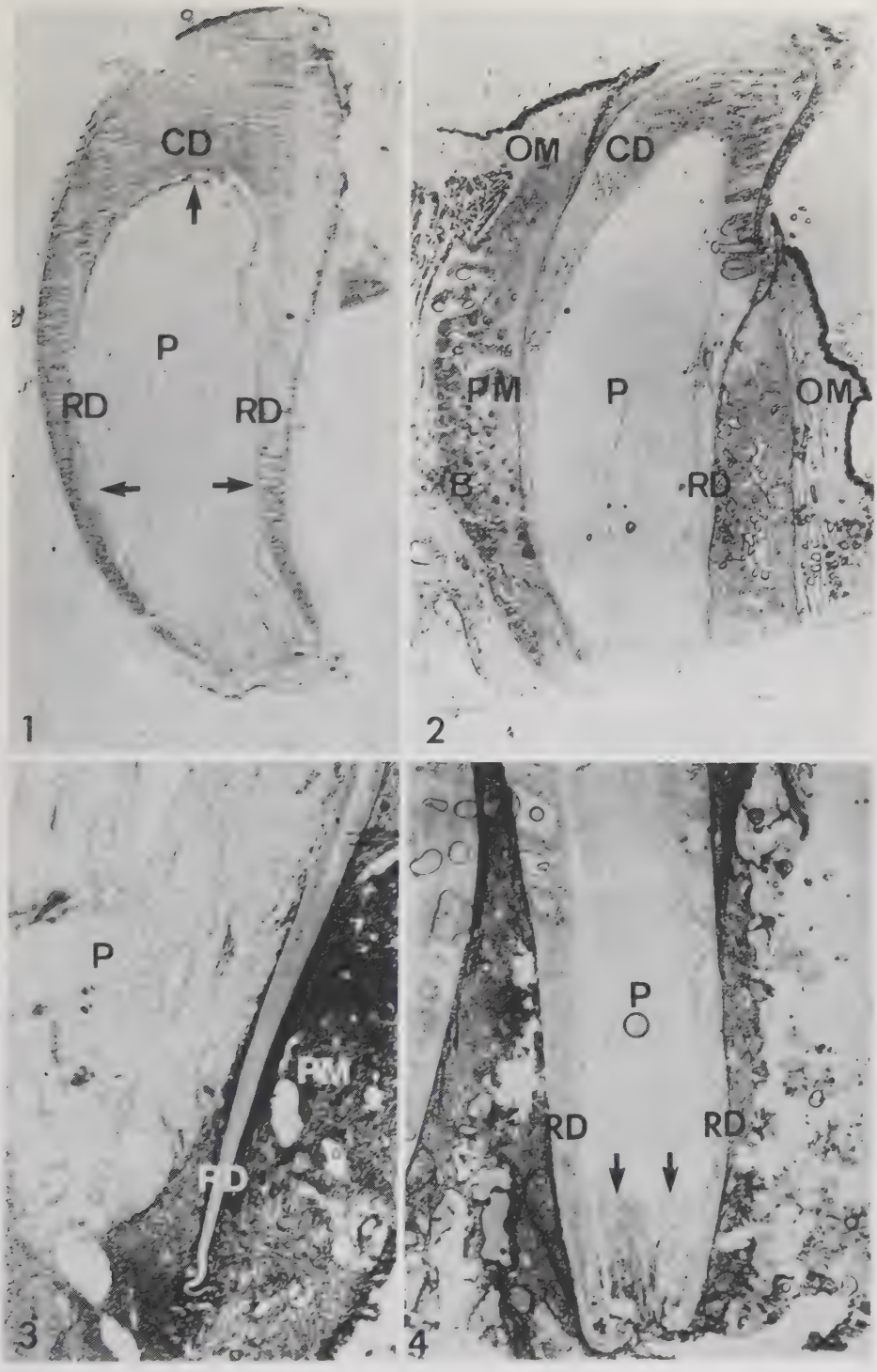

CD
P
RD
RD
1
OM
CD
PM
P
OM
B
RD
2
P
PM
RD
3
P
RD
RD
4

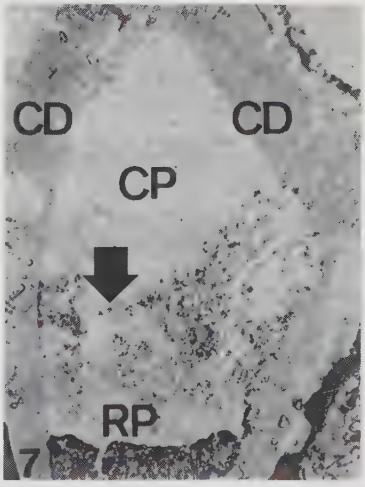
Fig. 5. Freeze-sectioned autotransplanted second maxillary incisor incubated for lactate dehydrogenase activity, 30 days postoperatively. Staining is noticed throughout the entire length of the pulp (P) with the highest activity coronally. B, bone. CD, coronal dentin. RD, root dentin. (Magnification, x 15.)

Fig. 6. Freeze-sectioned autotransplanted third mandibular incisor incubated for lactate dehydrogenase activity, 180 days postoperatively. Staining for enzyme activity is observed in all areas but the tissue is poor in cells in some areas (arrows). CP, coronal dentin. P, pulp. (Magnification, x 60.)

Fig. 7. Freeze-sectioned replanted second maxillary incisor incubated for lactate dehydrogenase activity, 180 days postoperatively. Staining for enzyme activity is observed in the root pulp (RP). The coronal pulp (CP) exhibits no staining for enzyme activity. CD, coronal dentin. (Magnification, x 60.)

CD
CD
P
B
B
RD
RD
5

CD
6


CD
CD
CP
RP
7

REFERENCES

1. Agnew, R.G., and Fong, C.C.: Histologic Studies on Experimental Transplantation of Teeth, Oral Surg. 9: 18-39, 1956.

2. Costich, E.R.: Basic Problems of Regeneration and Transplantation, Dental Clin. North Am.:513-526, 1962.

3. Nordenram, Å.: Autotransplantation of Teeth. A Clinical and Experimental Investigation, Acta Odontol. Scand. 21: Suppl. 33, 1963.

4. Öhman, A.: Healing and Sensitivity to Pain in Young Replanted Human Teeth. An Experimental, Clinical and Histological Study, Odontol. Tidskr. 73:166-227, 1965.

5. Monsour, F.N.T.: Pulpal Changes Following the Reimplantation of Teeth in Dogs. A Histological Study, Austr. Dent. J. 16:227-231, 1971.

6. Skoglund, A., Tronstad, L., and Wallenius, K.: A Microangiographic Study of Vascular Changes in Replanted and Autotransplanted Teeth of Young Dogs, Oral Surg. 45:17-28, 1978.

7. Ullberg, S.: Studies on the Distribution and Fate of S^{35}-Labeled Benzyl-penicillin in the Body, Acta Radiol. Suppl. 118, 1954.

8. Barka, T., and Anderson, P.J.: Histochemistry. Theory, Practice and Bibliography, New York, 1963. Harper and Row.

9. Seligman, A.M., Karnovsky, M.G., Wasserkrug, H.L., and Hanker, J.S.: Nondroplet Ultrastructural Demonstration of Cytochrome Oxidase Activity with a Polymerizing Osmiophilic Reagent, Diaminobenzidine (DAB), J. Cell. Biol. 38:1-14, 1968.

10. Mejàre, I., Hasselgren, G., and Hammarström, L.E.: Effect of Formaldehyde-containing Drugs on Human Dental Pulp Evaluated by Enzyme Histochemical Technique, Scand. J. Dent. Res. 83:29-36, 1975.

11. Rölling, I., Hasselgren, G., and Tronstad, L.: Morphologic and Enzyme Histochemical Observations on the Pulp of Human Primary Molars 3 to 5 Years after Formocresol Treatment, Oral Surg. 42:518-528, 1976.

12. Hasselgren, G., Larsson, Å., and Rundquist, L.: Pulpal Status after Autogenous Transplantation of Fully Developed Maxillary Canines, Oral Surg. 44:106-112, 1977.

III.

PULPAL CHANGES IN REPLANTED AND AUTOTRANSPLANTED IMMATURE TEETH OF DOGS

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ABSTRACT

The purpose of the present investigation was to study pulpal changes in replanted and autotransplanted immature teeth 10,30 and 180 days after the operation. At the end of the observation periods angiography was performed, and the dogs were sacrificed. The teeth were sectioned at 4 µm in a bucco-lingual direction and stained with hematoxylin and eosin, or according to the van Gieson or the Brown and Brenn methods. No difference was noted in the tissue reactions of the replanted and autotransplanted teeth. The pulp became necrotic during the first postoperative days. However, repair occurred through ingrowth of a well vascularized, cell-rich connective tissue that reached the pulp horn after approximately 30 days. In the majority of the teeth observed after 180 days the soft tissue of the pulp was markedly reduced in cells and blood vessels. Cell-containing atubular hard tissue occupied most of the original pulp cavity. In a few teeth the original pulp tissue seemed to have survived. In these teeth the pulp exhibited a normal appearance with an intact odontoblastic layer. Bacteria were not observed in the pulp or dentinal tubules in any of the teeth except in cervical dentinal cracks caused by the extraction procedures. Root resorption affected most teeth, and repair occurred parallell with the repair of the pulp.

INTRODUCTION

An important factor affecting the survival rate of replanted and autotransplanted teeth is the reaction of the pulp. (For review of the literature, see Natiella, Armitage and Greene,[1] Heithersay.[2]) Pulpal necrosis may give rise to periapical inflammation[3-7] and may also cause inflammatory root resorption.[8-11]

In a previous investigation the vascular system of the pulp of replanted and autotransplanted immature teeth was studied with a microangiographic technique.[12] Ten days postoperatively vessels filled with contrast medium were seen in the apical half of the pulp, and after 30 days, visible blood vessels were usually present in the whole pulp. Accordingly, the coronal half of the pulp was without vascular supply for days and weeks after the operation. In an enzyme histochemical study of replanted and autotransplanted immature teeth it was shown that the oxido-reductase enzyme activity of the pulp tissue gradually became weaker during the first 4 postoperative days.[13] After 10 days enzyme activity could be demonstrated only in the foraminal area of the pulp. 30 and 120 days postoperatively activity could again be demonstrated in the entire pulp of most teeth.

The purpose of the present investigations was to study the pulp tissue reactions in replanted and autotransplanted teeth during and after the time when pulpal revascularization supposedly occurs.[12]

MATERIAL AND METHODS

The experimental material consisted of sixty-five single-rooted teeth (incisors and first premolars) from nine healthy mongrel dogs approximately 4 months old at the beginning of the experiment. The root development was not completed at the time of replantation or autotransplantation. The dogs were anesthetized by intravenous administration of a barbiturate solution (Penthotal sodium ^{R}, (Abbott S.A. Brussels, Belgium). The teeth were extracted and either replanted or transplanted to the socket of the contralateral tooth. Surgical preparations of the sockets was not performed. During the extraoral time which did not exceed 10 minutes, the teeth were stored in sterile isotonic saline solution at room temperature. Intramuscu-

lar injections of penicillin (Suspenin Transin®, Kabi, Stockholm, Sweden) were given the day before the operation and the following 6 days (300 000 I.U. per day). Fixation of the teeth was not used. Five teeth were exfoliated during the first postoperative days and were discarded. The distribution of the rest of the experimental material is shown in Table I. The dogs were put on a diet of soft food for 2 weeks or until the time of sacrifice. The observation periods chosen were 4,10,30 and 180 days (Table II).

At the end of the observation periods, the animals were anesthetized by intravenous administration of a barbiturate solution (Mebumal Vet®, ACO, Solna, Sweden). Angiography of the jaws and teeth was performed by a method described earlier.[12,14]

The jaws were excised, fixed in 4 per cent neutral-buffered formaldehyde (pH 7.4) and demineralized in 5.2 per cent nitric acid. Longitudinal paraffin serial sections were taken from the teeth at 4 µm in a bucco-lingual direction, and stained with hematoxylin and eosin or according to the van Gieson or the Brown and Brenn methods.

For control purposes thirty-six intact teeth, one from each quadrant of the jaws, were sectioned. The Brown and Brenn method was controlled by the use of tissue sections with known bacterial invasion.

RESULTS

Control teeth

A normal tissue with an intact odontoblastic layer and a uniform predentin zone was present in the whole pulp cavity (Fig. 1). A majority of the blood vessels contained contrast medium, sometimes mixed with erythrocytes. In the dogs sacrificed 4,10, and 30 days after the operation, the root development was not completed. In the

dogs sacrificed after an observation period of 180 days a bulky root tip containing channels forming a root canal delta was seen. Bacteria were not observed in the dentinal tubules or the pulp. Internal or external root resorption had not occurred.

Experimental teeth

In some of the teeth slight damage due to the extraction procedures was evident, leading to cracks with exposure of the dentin in the cervical areas. Bacteria were seen in these cracks, but otherwise only in plaque on tooth surfaces. No difference was noted in the tissue reactions of the replanted and autotransplanted teeth.

Four days postoperatively (thirteen teeth). A well vascularized and well stained connective tissue was observed only in the foraminal region of the pulp. As a rule this tissue extended somewhat further coronally near the dentin than centrally in the pulp, and one or a few layers of cells were lining the root canal walls (Figs. 2a and c). In the rest of the pulp the various components of the tissue including the odontoblastic layer were discernible, but they were clearly less stained than near the foramen (Figs. 2a,b and c). In three teeth blood vessels containing contrast medium were observed in the root pulp and in areas of the coronal pulp, i.e., in the weakly stained areas of the tissue (Fig. 2a). Extravasated erythrocytes were seen in all teeth adjacent to blood vessels in the apical part of the pulp (Fig. 2c). Brown pigment was evident in the whole pulp. Internal or external root resorption was not found.

Ten days postoperatively (fourteen teeth). A cell-rich, well vascularized connective tissue was observed in the apical part of the pulp of all teeth (Figs. 3a and c). At the dentinal walls near the foramen a thin hard tissue matrix, occasionally containing cell-inclusions, was seen. The hard tissue matrix was lined by one or a few layers of cells (Fig. 3d). In the middle and coronal

part of the pulp the morphologic structures were weakly stained and only vaguely discerned (Figs. 3a and b). Blood vessels filled with contrast medium and/or erythrocytes were present in the apical part of the pulp. Many of the vessels reached into the weakly stained tissue further coronally (Fig. 3a). In the border zone between the vascularized and non-vascularized areas of the pulp, large accumulations of extravasated erythrocytes and occasionally inflammatory cells were evident (Fig. 3a). In the coronal half of the pulp blood vessels with contrast medium or erythrocytes were not found. Brown pigment was seen in the whole pulp (Fig. 3b).

Thirty days postoperatively (eighteen teeth). A normal pulp was observed in two teeth (Fig. 4a). An odontoblastic layer was present throughout the entire length of the pulp in these teeth, and tubular dentin had formed postoperatively in all areas. In the remaining sixteen teeth accumulations of extravasated erythrocytes and/or necrotic areas bordered by inflammatory cells were noted in the coronal pulp (Figs. 4a and 5a). Further apically, a well vascularized cell-rich connective tissue with scattered macrophages was seen. The majority of the teeth in this group showed a small amount of postoperatively formed tubular dentin in the most apical part of the root canal, and in these teeth a root tip with channels containing soft tissue had formed (Figs. 4a and 5a). Further coronally a cell-containing, atubular hard tissue had been laid down at the root canal walls, and in some teeth such a tissue was also observed as a layer between pre- and postoperatively formed tubular dentin in the apical area (Fig. 5a). Both the cell-containing hard tissue and the dentin was lined by one or a few layers of cells (Figs. 4a and d). A tissue resembling immature bone had formed in the central areas of the pulp of four teeth (Figs. 4b and d). In another two teeth minor internal resorption areas were noted in the coronal pulp. Apposition of hard tissue had occurred in the resorption lacunae in regions without inflammation (Figs. 5a and b). External

root resorption lacunae were found in all teeth, usually in the apical half of the root (Fig. 5a). In two teeth the resorption process had reached the pulp cavity. In one of these teeth a fracture of the root had occurred. In the other teeth the resorption lacunae were small and usually filled with hard tissue (Fig. 4c). Only rarely were multi-nucleated cells detected.

One hundred and eighty days postoperatively (fifteen teeth). In two teeth, a pulp with normal morphology was observed. An odontoblastic layer was seen lining tubular dentin that had formed postoperatively on the root canal walls in all areas of the pulp. Cell-containing hard tissue was not found in the pulp of these teeth. In the remaining thirteen teeth a connective tissue poor in cells and blood vessels was noted throughout the pulp (Figs. 6a and b). Cells reminding of odontoblasts were not observed in these teeth. Large hard tissue deposits, formed after the operation were seen on the root canal walls (Figs. 6a, b and c). The tissue contained cells and had a lamellar arrangement. In a majority of the teeth a slight amount of dentin had formed postoperatively in the foraminal region. Upon this dentin large amounts of cell-containing, atubular hard tissue had been laid down. A bone-like tissue had formed centrally in the pulp and in six teeth this tissue was continuous with the alveolar bone through a wide open apical foramen (Fig. 6b). Between the bony trabeculae a bone marrow-like tissue was noted. Macrophages were scattered in the whole pulp of nine teeth (Fig. 6d). Other inflammatory cells were not observed. Small internal resorption areas with apposition of hard tissue were seen in two teeth. External root resorption lacunae with apposition of hard tissue were found in all teeth.

DISCUSSION

As was mentioned in the introduction, the results of two previous studies[12,13] suggested that the pulp of replanted and autotransplanted teeth with partly formed roots became necrotic after the operation, and that revascularization then occurred during a 30-day period. Further observations revealed that 180 days postoperatively the number of pulpal blood vessels, and especially the number of smaller branches, was markedly reduced as compared with the teeth observed 30 days after the operation. In the present study it was possible to obtain a more detailed picture of the reaction of the pulp of replanted and autotransplanted teeth by a combination of angiographic and histologic techniques.

Four days postoperatively the normal morphologic structure of the pulp was recognizable. However, the tissue components were weakly stained, and brown pigment was observed in the tissue. These findings as well as cellular details, indicated that the tissue was in poor condition, and confirmed the observations in an enzyme histochemical study, in which the oxido-reductase activity had decreased markedly during the same observation period in all areas of the pulp except near the foramen.[13] In the foraminal area pulpal repair was evident,[13] and in the present study a well vascularized, cell-rich tissue was seen.

Ten days postoperatively a cell-rich, highly vascular connective tissue was observed in the apical third of the pulp. In the middle and coronal areas the tissue showed an even more ghost-like appearance than after 4 days, and the components of the tissue were hardly discernible. Thus, the tissue in these areas now showed definite signs of necrosis.[14] This is again in agreement with the findings of the enzyme histochemical study,[13] and of great importance, it is also in agreement with histologic findings in human teeth.[15]

It is evident that the repair processes in the pulp had advanced between 4 and 10 days postoperatively, although not to such an extent as was indicated with microangiography.[12] This is in accordance with what might be expected and also with what was seen histochemically that the ingrowth and recanalization of blood vessels dominated the early phase of the tissue repair.[13] In the border zone between the vascularized and nonvascularized areas of the pulp large accumulations of extravasated erythrocytes were a conspicuous finding. Excessive extravasation of erythrocytes into areas of ischemic necrosis has been observed also in other parts of the body, for example after infarction due to an embolus in a large artery, when revascularization and reflow through the vessels occur.[16]

Thirty days after the operation the repair had usually reached the pulp horn. However, small areas of necrotic tissue was seen in the coronal pulp of some teeth, and adjacent to the necrotic areas inflammatory cells were observed. However, inflammation was not a prominent feature of the repair processes in the present study. According to previous investigations[17-19] sterile necrotic tissue gives rise to only a mild inflammatory reaction in adjacent tissue. In the present study care was taken to avoid infection. The extra-oral time was kept as short as possible, the teeth were stored in sterile saline solution during this time, and antibiotics were used. The precautions appeared to have been effective since microorganisms were not found in necrotic pulp tissue in any of the teeth.

In the teeth observed 180 days postoperatively the number of blood vessels and cells of the pulp was markedly reduced as compared to the 30-day teeth. Changes of this nature have been reported to occur also in other parts of the body subjected to ischemic necrosis, for example in the heart after a coronary infarction.[21] Removal of necrotic heart muscle and replacement by a cell-rich connective

tissue is seen between 7 and 14 days. Then the tissue becomes less rich in cells and blood vessels during the next 30 days and a scar is formed.[21]

In clinical and experimental studies it has been found that the pulp of replanted and autotransplanted teeth often obliterates.[3,4,15,20,22-29] In the present investigation a small amount of hard tissue matrix had formed 10 days postoperatively in regions undergoing repair. In the majority of the teeth observed 180 days postoperatively large areas of the original pulpal cavity were filled with hard tissue resembling bone or cementum. Furthermore odontoblasts were not observed. In a few teeth, however, a normal pulp tissue with an intact odontoblastic layer was seen. In these teeth, a normal amount of tubular dentin had formed postoperatively, and cell-containing hard tissue was not found. Consequently, it appeared that in these teeth the original pulp tissue had survived the operation. According to the previous enzyme histochemical study[13] oxido-reductase activity could be demonstrated in the pulp tissue for a period of 4 days after replantation or autotransplantation. It is conceivable, therefore, that the original pulp tissue may survive if revascularization occurs within this time period. The findings of a partly restored circulatory system throughout the entire length of the pulp in some 4-day teeth in the present and a previous study[12] suggested that this is possible.

Failures due to root resorption are often seen after replantation and autotransplantation of teeth.[1-4,6,20,22-24] In the present study internal root resorption was only rarely observed. External root resorption, however, affected most of the teeth and started usually as early as between 4 and 10 days postoperatively. However, the resorption was arrested and repair occurred approximately parallell with the repair of the pulp.

In conclusion, the present study confirmed that the pulp of replanted and autotransplanted immature teeth as a rule became necrotic after the operation. Repair occurred through ingrowth of a well vascularized, cell-rich connective tissue that reached the pulp horn after approximately 30 days. 180 days after the operation the pulp tissue was markedly reduced in cells and blood vessels, and a tissue that resembled bone or cementum occupied most of the original pulp cavity. In a few teeth, however, the original pulp tissue appeared to have survived the operation. In these teeth the pulp had a normal appearance with an intact odontoblastic layer, and no atubular hard tissue was observed.

Table I. Distribution of experimental material

	No. of teeth	First incisor	Second incisor	Third incisor	First premolar
Maxilla					
Replanted teeth	13	4	2	2	5
Transplanted teeth	14	4	6		4
Mandible					
Replanted teeth	14	5	6	3	0
Transplanted teeth	19	4	6	2	7
Total	60	17	20	7	16

Table II. Distribution of experimental material according to observation periods

	No. of teeth	Observation periods			
		4 days	10 days	30 days	180 days
Replanted teeth	27	5	5	10	7
Transplanted teeth	33	8	9	8	8
Total	60	13	14	18	15

LEGENDS

Fig. 1. Control tooth (maxillary third premolar). A normal pulp tissue with an intact odontoblastic layer and an even predentin zone is seen. The majority of the blood vessels are filled with contrast medium. B, bone. CD, coronal dentin. P, pulp. RD, root dentin. (Hematoxylin and eosin stain. Magnification, x 10.)

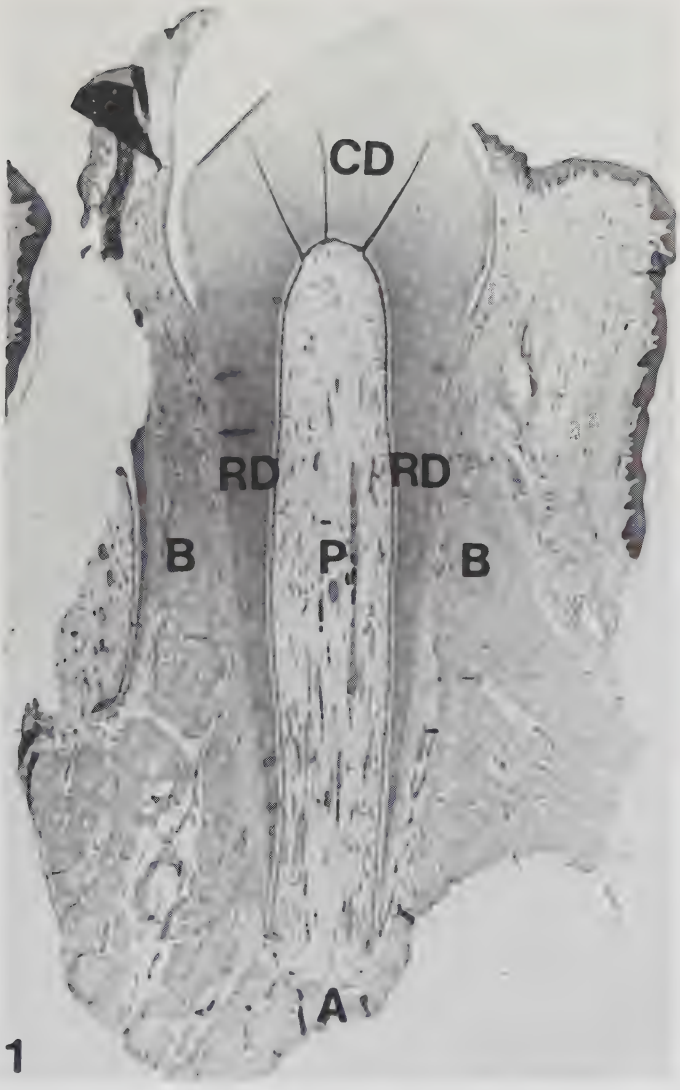

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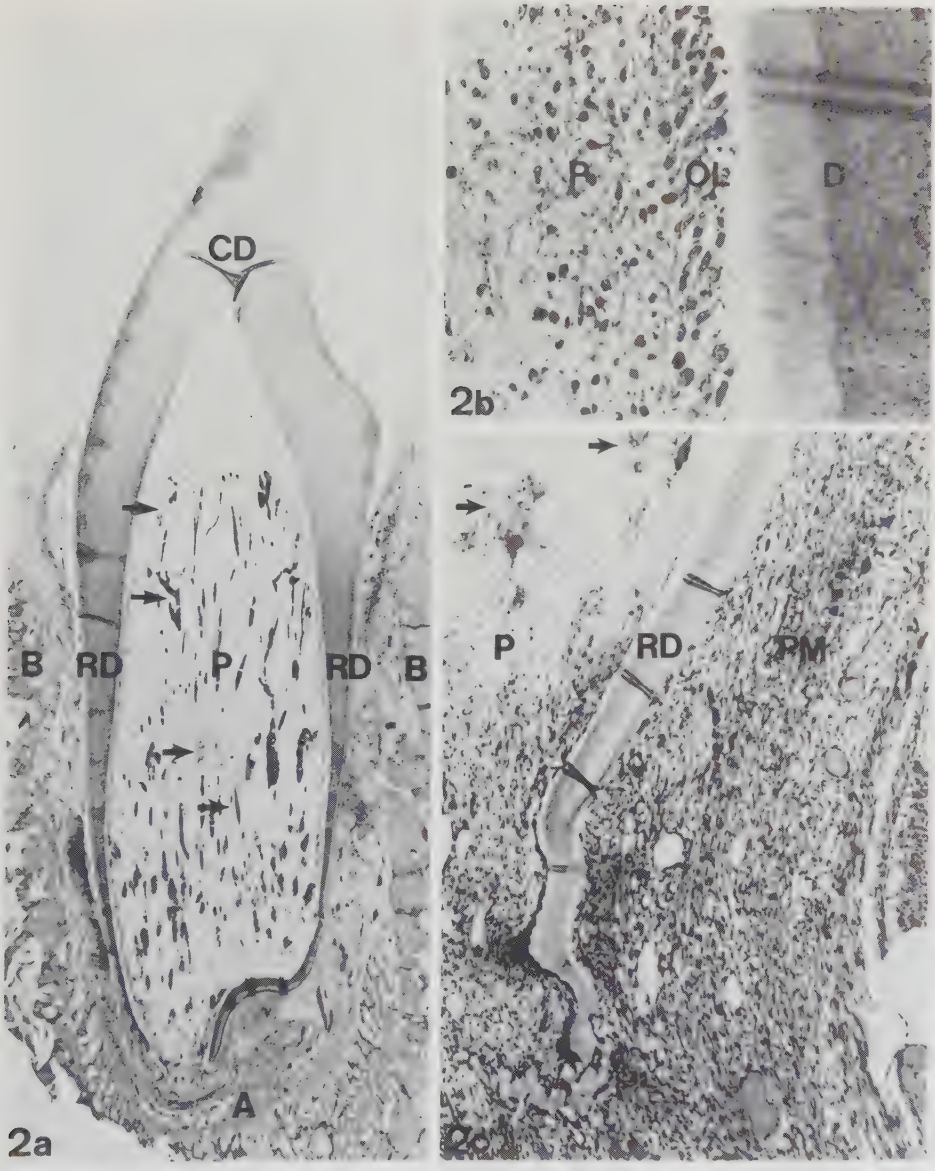
Fig. 2a. Autotransplanted second maxillary incisor, 4 days postoperatively. Connective tissue of normal appearance is observed in the foraminal region of the pulp. Blood vessels containing contrast medium (arrows) and erythrocytes extend into weakly stained tissue in the root pulp. Accumulations of extravasated erythrocytes are observed adjacent to some vessels. A, apex. B, bone. CD, coronal dentin. RD, root dentin. P, pulp. (Hematoxylin and eosin stain. Magnification, x 15.)

Fig. 2b. Detail from Fig. 2a, middle portion of the pulp. Many of the cells exhibit a roundish appearance. The odontoblastic layer is clearly distinguishable. D, dentin. OL, odontoblastic layer. P, pulp. (Magnification, x 400.)

Fig. 2c. Replanted third mandibular incisor, 4 days postoperatively, apical region. A connective tissue of normal appearance is seen only in the foraminal area of the pulp. A few rows of cells are lining the root canal walls in this region. Extravasated erythrocytes are observed adjacent to some contrast-filled vessels (arrows). A few of these vessels extend into the weakly stained tissue in the region further coronally. P, pulp. PM, periodontal membrane. RD, root dentin. (Hematoxylin and eosin stain. Magnification, x 60.)


CD
B
RD
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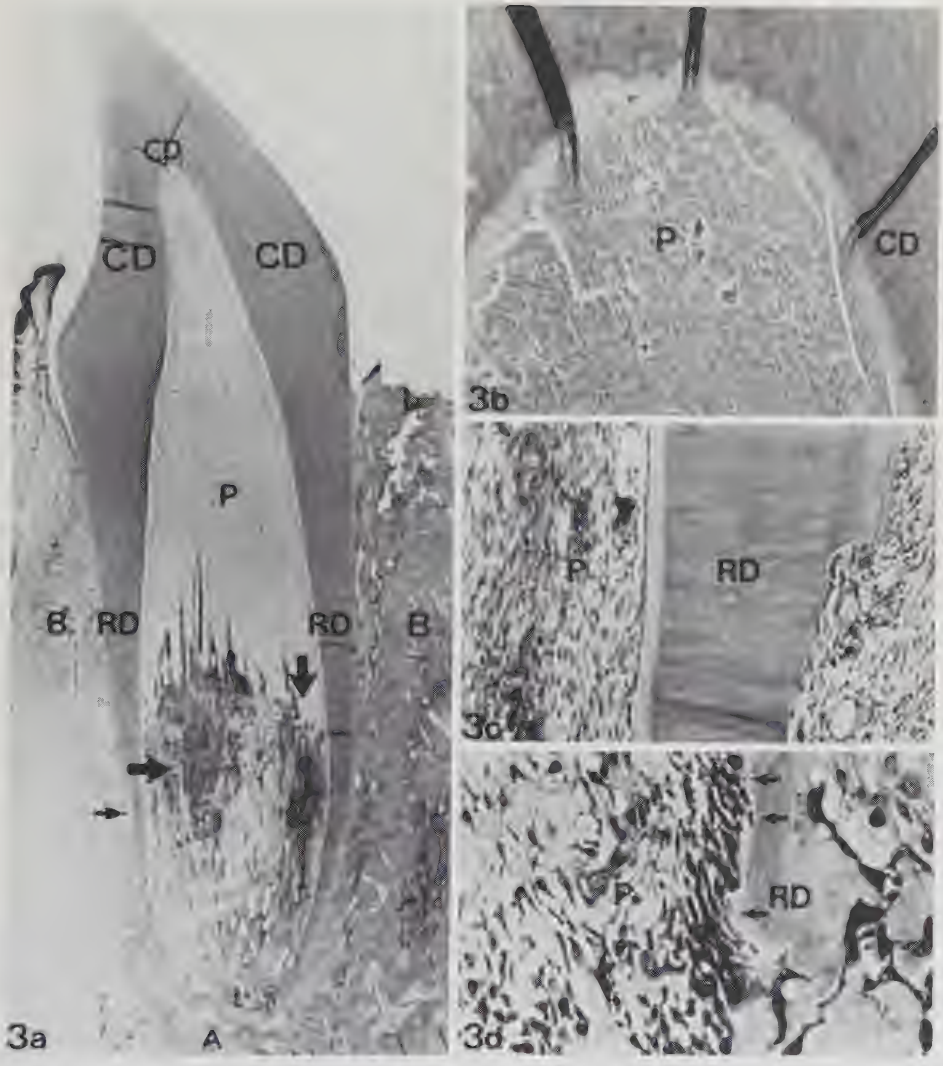
RD
B
A
2a
P
OL
2b
D
P
RD
PM
2c

Fig. 3a. Autotransplanted second mandibular incisor, 10 days postoperatively. Connective tissue of normal appearance is observed in the apical part of the pulp. Blood vessels extend into the area further coronally where known morphologic structures are only vaguely discerned. In the border area between vascularized and nonvascularized tissue large accumulations of extravasated erythrocytes are seen (thick arrows). External root resorption lacunae are present in the apical half of the root (thin arrows). A, apex. CD, coronal dentin. RD, root dentin. P, pulp. (Hematoxylin and eosin stain. Magnification, x 15.)

Fig. 3b. Detail from Fig. 3a, coronal area of the pulp. The morphologic structures of the tissue are almost indistinguishable. Brown pigment is seen. CD, coronal dentin. P, pulp. (Magnification, x 100.)

Fig. 3c. Replanted first mandibular incisor, 10 days postoperatively, apical third of the root. A cell-rich connective tissue containing accumulations of extravasated erythrocytes is seen in the pulp. Root resorption lacunae with multi-nucleated cells are observed at the external surface of the root (at right). RD, root dentin. P, pulp. PM, periodontal membrane. (Hematoxylin and eosin stain. Magnification, x 100.)

Fig. 3d. Detail from Fig. 3a, apical region. Rows of cells (black arrows) are seen next to a thin layer of hard tissue matrix at the root canal wall. P, pulp. RD, root dentin. (Magnification, x 400.)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CD
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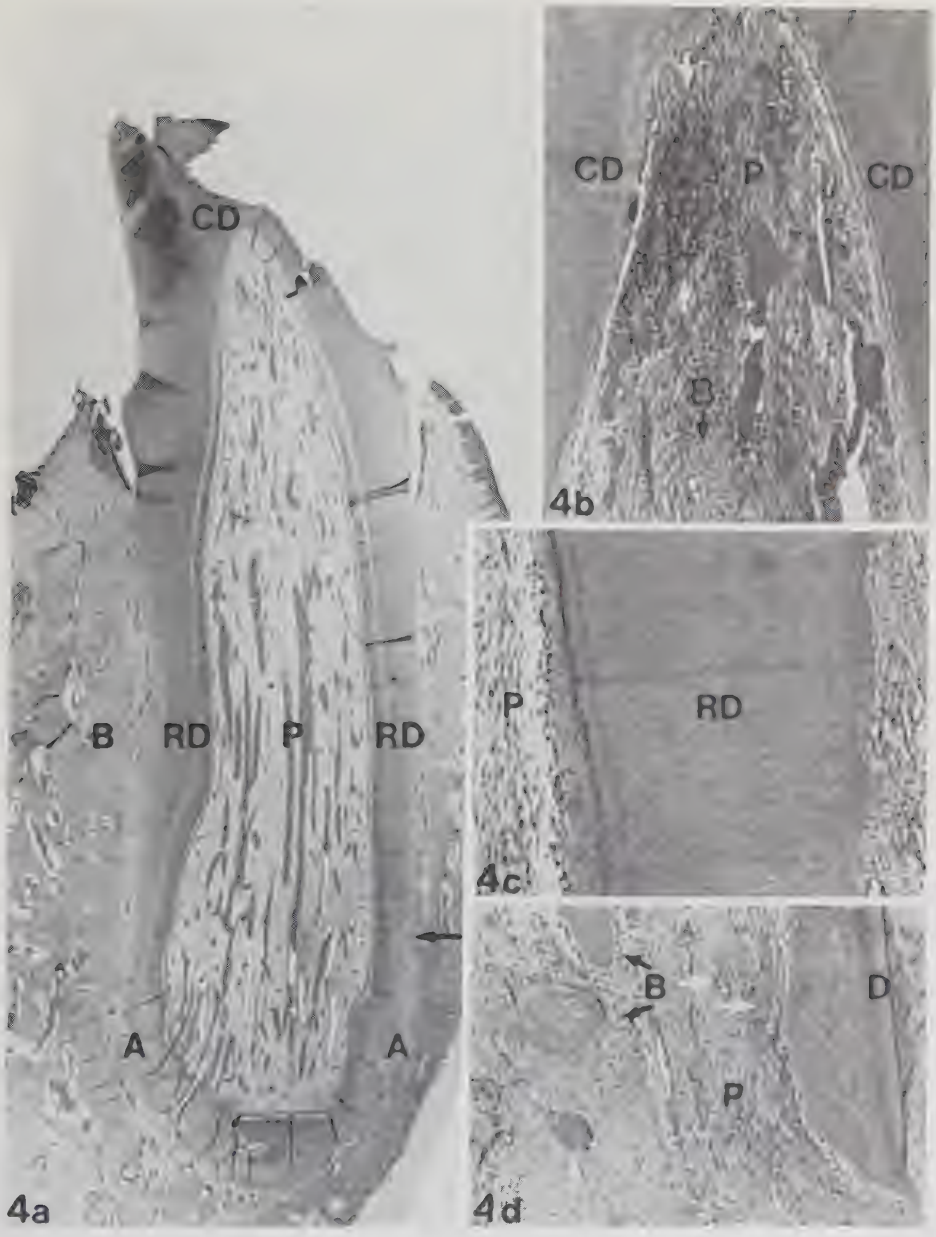
3d

Fig. 4a. Replanted second maxillary incisor, 30 days postoperatively. A connective tissue of normal appearance and with blood vessels containing contrast medium is observed throughout the pulp. Tubular dentin has been laid down postoperatively at the root canal walls, and is lined by odontoblasts. External root resorption lacunae are observed (arrows). A root tip has formed postoperatively with channels containing soft tissue. A, apex at the time of operation. B, bone. CD, coronal dentin. P, pulp. RD, root dentin. (Hematoxylin and eosin stain. Magnification, x 15.)

Fig. 4b. Replanted second maxillary incisor (contralateral to the tooth shown in Fig. 4a), 30 days postoperatively, coronal area of the pulp. A cell-rich well vascularized tissue is seen, in which bone-like tissue has been laid down (black arrows). Numerous extravasated erythrocytes are observed in conjunction with contrast-filled blood vessels (white arrows). B, bone-like tissue. CD, coronal dentin. P, pulp. (Hematoxylin and eosin stain. Magnification, x 100.)

Fig. 4c. Same tooth as in Fig. 4b, middle portion of the root. In the pulp a cell-rich connective tissue is observed. A cellular hard tissue has been laid down at the root canal wall and is lined by a few layers of cells. External root resorption lacunae with apposition of hard tissue are observed (to the right). P, pulp. RD, root dentin. (Magnification, x 100.)

Fig. 4d. Same tooth as in Figs. 4b and 4c, apical portion of the pulp. Contrast-filled blood vessels (at white arrows) are seen in the connective tissue. Cell-containing, atubular hard tissue has formed at the root canal wall and centrally in the pulp. A, apex. B, bone-like tissue. D, dentin. P, pulp. (Magnification, x 100.)


CD
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D
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4d

Fig. 5a. Autotransplanted first maxillary incisor, 30 days postoperatively. Connective tissue is observed in the root pulp. Blood vessels containing contrast medium are seen throughout the entire length of the root pulp (thick, black arrows). In the pulp horn necrotic tissue is observed bordered by inflammatory cells (thin black arrows). Small internal root resorption lacunae are seen adjacent to the inflamed area of the pulp (at right, thin white arrows). Repair has occurred to the left (thick white arrows). Slight damage due to the extraction procedures is evident in the cervical area of the root. A small external root resorption lacunae is seen in the middle portion of the root (to the right). A root tip with channels containing soft tissue has formed postoperatively. CD, coronal dentin. P, pulp. RD, root dentin. (Hematoxylin and eosin stain. Magnification, x 15.)

Fig. 5b. Detail from Fig. 5a, coronal pulp. Apposition of hard tissue is observed in internal root resorption lacunae adjacent to uninflamed connective tissue. An area with inflammation is seen to the right. (Magnification, x 100.)

Fig. 5c. Detail from Fig. 5a, central area of the coronal pulp. Inflammatory cells are observed. (Magnification, x 400.)

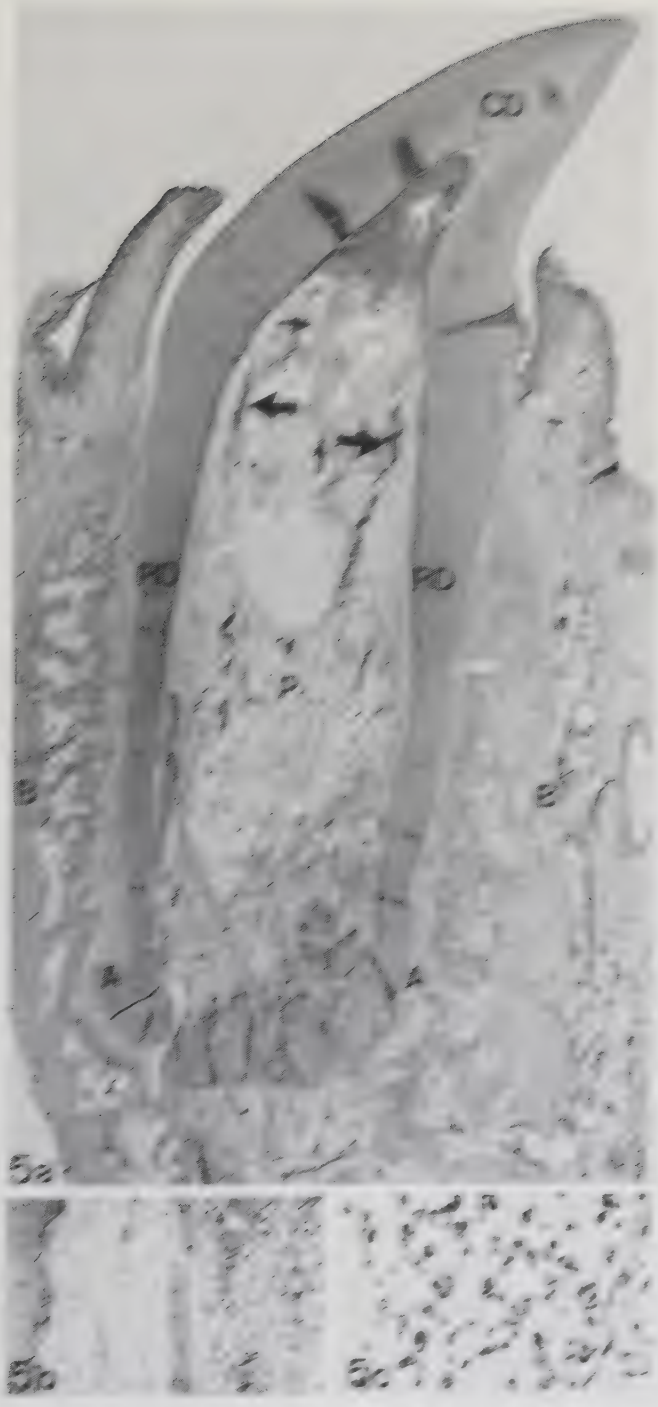
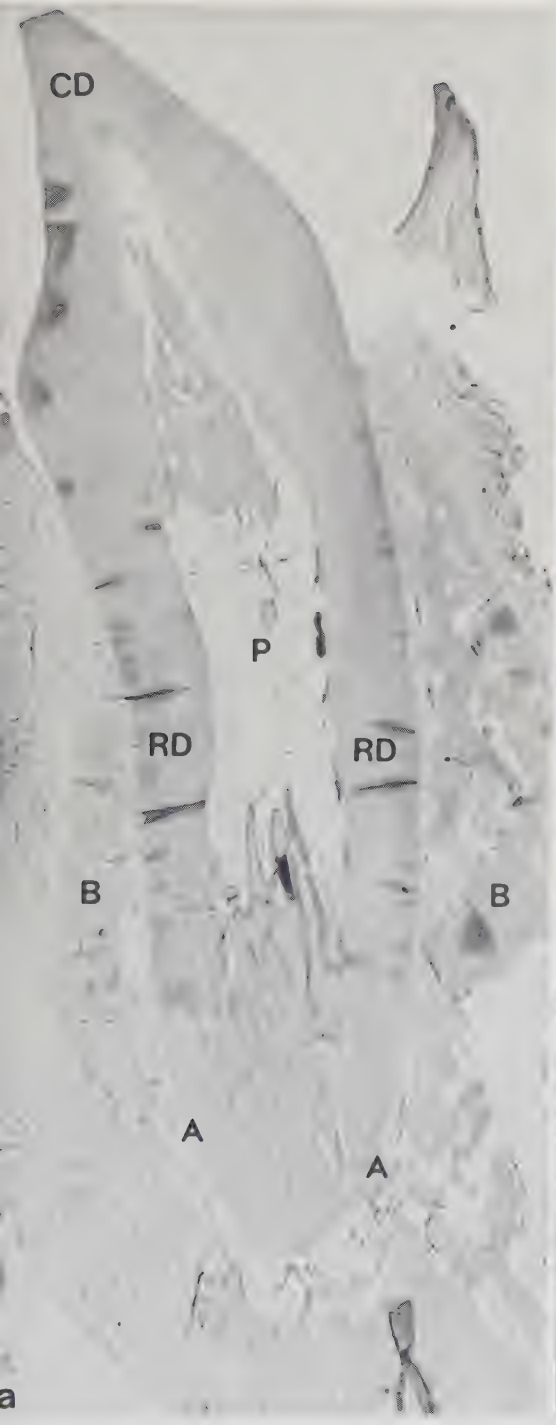

PD
PD
P
B
B
A
A
5a
5b
5c

Fig. 6a. Autotransplanted second mandibular incisor, 180 days postoperatively. Connective tissue poor in cells and blood vessels is observed throughout the entire length of the pulp. The degree of root development at the time of operation is visible (A). Excessive hard tissue formation is observed in the pulp cavity. An odontoblastic layer is not seen. B, bone. CD, coronal dentin. P, pulp. RD, root dentin. (Hematoxylin and eosin stain. Magnification, x 15.)

Fig. 6b. Autotransplanted second maxillary incisor, 180 days postoperatively. A lamellar arranged cellular hard tissue is seen along the dentinal wall. Intrapulpal bone which is continuous with the socket wall through the open apical foramen, is seen. A, apex at the time of operation. B, bone. CD, coronal dentin. IB, intrapulpal bone. P, pulp. RD, root dentin. (Hematoxylin and eosin stain. Magnification, x 15.)

Fig. 6c. Detail from Fig. 6b, apical area. Cellular hard tissue has formed postoperatively at the root canal wall. External root resorption lacunae with apposition of hard tissue are seen at the apex. P, pulp. RD, root dentin. (Magnification, x 100.)

Fig. 6d. Detail from Fig. 6b. A fibrous connective tissue extremely poor in cells is observed. Accumulations of macrophages are seen in this tissue (black arrows). Blood vessels filled with contrast medium are also observed (white arrows). (Magnification, x 400.)


CD
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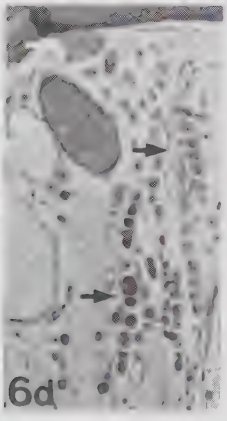
6d

REFERENCES

1. Natiella, J.R., Armitage, J.E., and Greene,G.W.: The Replantation and Transplantation of Teeth, Oral Surg. 29:397-419, 1970.

2. Heithersay, G.S.: Replantation of Avulsed Teeth. A Review, Aust. Dent. J. 20:63-72, 1975.

3. Hansen, J., and Fibaek, B.: Clinical Experience of Auto- and Allotransplantation of Teeth, Int. Dent. J. 22:270-285, 1972.

4. Lovius, B.B.J., Atherton, J.D., Wynne, T.H.M., and Finch, L.D.: Autogenous Tooth Transplantation: a Clinical and Histological Investigation, Br. J. Orthod. 1:27-33, 1972.

5. Woehrle, R.R.: Cementum Regeneration in Replanted Teeth with Differing Pulp Treatment, J. Dent. Res. 55: 225-238, 1976.

6. Nasjleti, C.T., Caffesse, R.G., and Castelli, W.A.: Replantation of Mature Teeth without Endodontis in Monkeys, J. Dent. Res. 57:650-658, 1978.

7. Barbakow, F.H., Austin, J.C., and Cleaton-Jones, P.E.: Experimental Replantation of Root-canal-filled and Untreated Teeth in the Vervet Monkey, J. Endod. 3:89-93, 1977.

8. Knight, M.K., Gans, B.J., and Calandra, J.C.: The Effect of Root Canal Therapy on Replanted Teeth of Dogs, Oral Surg. 18:227-242, 1964.

9. Andreasen, J.O., and Hjørting-Hansen, E.: Replantation of Teeth. 1. Radiographic and Clinical Study of 110 Human Teeth Replanted after Accidental Loss, Acta Odontol. Scand. 24:263-286, 1966.

10. Rotschild,D.L., Goodman, A.A., and Blakey, K.R.: A Histologic Study of Replanted and Transplanted Endodontically and Nonendodontically Treated Teeth in Dogs, Oral Surg. 28:871-876, 1969.

11. Andreasen, J.O.: Effect of Pulpal Necrosis upon Periodontal Healing after Surgical Injury in Rats, Int. J. Oral Surg. 2:62-68, 1973.

12. Skoglund, A., Tronstad, L., and Wallenius, K.: A Microangiographic Study of Vascular Changes in Replanted and Autotransplanted Teeth of Young Dogs, Oral Surg. 45:17-28, 1978.

13. Skoglund, A., Hasselgren, G., and Tronstad, L.: Oxidoreductase Activity in the Pulp of Replanted and Autotransplanted Teeth in Young Dogs. Accepted for publication. Oral Surg.

14. Hasselgren. G., Larsson, Å., and Rundquist, L.: Pulpal Status after Autogenous Transplantation of Fully Developed Maxillary Canines, Oral Surg. 44:106-112, 1977.

15. Öhman, A.: Healing and Sensitivity to Pain in Young Replanted Human Teeth. An Experimental, Clinical and Histologic Study, Odont. T. 73:166-227, 1965.

16. Robbins, S.L.: Pathologic Basis of Disease, W.B. Saunders Co., Philadelphia, London, Toronto 1974, p. 1507.

17. Torneck, C.D.: Reaction of Rat Connective Tissue to Polyethylene Tube Implants, Oral Surg. 24:674-683, 1967.

18. Bergenholz, G.: Microorganisms from Necrotic Pulp of Traumatized Teeth, Odont. revy 25:347-358, 1974.

19. Makkes, D.C., Thoden van Velzen, S.K., and Wesselink, P.R.: The Response of the Living Organism to Dead and Fixed Dead Tissue, J. Endod. 4:17-21, 1978.

20. Oksala, E.: Autotransplantation of Vital Maxillary Canines. A Clinical and Radiographic Study, Proc. Finn. Dent. Soc. 70: Suppl., 1974.

21. Anderson, W.A.D., and Scotti, T.M.: Synopsis of Pathology, 1972, St. Louis, The C.V. Mosby Co., p. 347-351.

22. Nordenram, Å.: Autotransplantation of Teeth. A Clinical and Experimental Investigation, Acta Odontol. Scand. 22: Suppl. 33, 1963.

23. Moss, J.P.: Autogenous Transplantation of Maxillary Canines, J. Oral Surg. 26:775-783, 1968.

24. Andreasen, J.O., Hjørting-Hansen, E., and Jølst, O.: A Clinical and Radiographic Study of 76 Autotransplanted Third Molars, Scand. J. Dent. Res. 78:512-523, 1970.

25. Singh, K.K., and Dudani, J.C.: Autogenous Transplantation of Developing Mandibular Third Molars, J. Indian Dent. Assoc. 42:199-212, 1970.

26. Slagsvold, O.: Autotransplantation of Premolars in Cases of Missing Anterior Teeth, Trans. Eur. Orthod. Soc. 46th Congr., pp. 473-483, 1970.

27. Cook, R.M.: The Current Status of Autogenous Transplantation as Applied to the Maxillary Canine, Int. Dent. J. 22:286-300, 1972.

28. Anderson, A.W., Sharav, Y., and Massler, M.: Reparative Dentine Formation and Pulp Morphology, Oral Surg. 26:837-847, 1968.

29. Agnew, R.G., and Fong, C.C.: Histologic Studies on Experimental Transplantation of Teeth, Oral Surg. 9: 18-39, 1956.

IV

VASCULAR CHANGES IN REPLANTED AND AUTOTRANSPLANTED APICOECTOMIZED MATURE TEETH OF DOGS

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Running title: Vascular changes in mature tooth grafts.

Key words: apicoectomy, microcirculation, microradiography, tooth reimplantation, tooth transplantation.

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ABSTRACT

The purpose of the present investigation was to study whether intentional fracturing of the root tip at the time of replantation or autotransplantation of mature teeth might improve the revascularization capacity of the pulp. The vascular system in apicoectomized and nonapicoectomized replanted and autotransplanted mature dog teeth was studied 10, 30 and 120 days postoperatively by means of a barium sulfate injection medium combined with microradiography. Pulpal revascularization occurred during a 120-day period in eight out of nine apicoectomized teeth. The pulp of the nonapicoectomized teeth did not revascularize.

INTRODUCTION

Pulp reactions following replantation and autotransplantation of fully developed teeth have been studied in clinical and experimental investigations[1-16]. Some authors have claimed that healing of the pulp is possible, and endodontic treatment as a routine procedure postoperatively has therefore not been recommended[3,5-10,12]. According to other researchers the pulp in such teeth necrotizes, except on rare occasions[1,11,13,14,16,17], and therefore endodontic treatment should be performed as soon as possible after the operation[1,13,18,19]. Some investigators have accidentally or deliberately fractured the root tip of mature teeth before replantation or autotransplantation[2,11,12,15]. However, it has not been possible to ascertain whether or not this procedure has any noticeable effect on the healing of the pulp. As examples of conflicting results Flath[2] has reported regained sensibility in eight out of nine apicoectomized transplants, while Kallionemi and Oksala[15] has recorded signs of vitality in only one out of eight such teeth.

The purpose of the present investigation, therefore, was

to study by means of a microangiographic technique whether intentional fracturing of the root tip at the time of replantation or autotransplantation might improve the revascularization capacity of the pulp of mature teeth.

MATERIAL AND METHODS

The experimental material consisted of 52 single-rooted teeth (incisors and first premolars) in five adult mongrel dogs. In all teeth the apical foramina were narrow indicating that the root development was completed (Fig. 1). The experimental teeth were extracted. Sixteen teeth were then replanted and 10 autotransplanted to the socket of the contralateral tooth after apicoectomy performed with a wire cutter. About one fourth of the length of the root was removed. The contralaterals were likewise replanted or autotransplanted but without apicoectomy. The dogs were anesthetized during these procedures by intravenous administration of a barbiturate solution (Penthotal sodium ®, Abbott, Brussels, Belgium). Fixation devices were not used. The extraoral time did not exceed 10 minutes, and during this period the teeth were stored in sterile isotonic saline solution at room temperature. The sockets were not prepared surgically. Penicillin (Penicillin prokain Vet. ®, Novo, Copenhagen, Denmark)(600.000 I.U. per day) was given intramuscularly the day before the operation and the following seven days.

The observation periods were 10, 30 and 120 days. The distribution of the experimental material according to observation periods is seen in Table I.

At the end of the observation periods, the dogs were anesthetized by intravenous administration of a barbiturate solution (Mebumal Vet ®, ACO, Solna, Sweden) and subcutaneous injections of Heparin ® (Vitrum, Stockholm, Sweden) 500 I.U. per kilogram of body weight, were given.

The common carotid arteries were cannulated and perfused with a warm (37°C) barium sulfate injection medium consisting of Micropaque® (Nicholas, Slough, Bucks, England) (60 percent) and isotonic saline solution (40 percent). The injection was performed by means of a pump at a pressure slightly above the systolic blood pressure (170 mm. Hg). The jugular veins were cut. When the blood vessels under the tongue were filled with Micropaque® and there was a flow of contrast medium from the jugular veins, the dogs were injected with a warm (37°C) solution of Micropaque® (60 percent) and 4 percent neutral buffered formaldehyde (40 percent) until muscle fasciculations were noted, and the dogs were sacrified during this procedure.

The jaws were then excised and fixed in 4 percent neutral-buffered formaldehyde. After demineralization in 5 percent nitric acid, longitudinal slices about 1 mm thick were cut from the middle portion of the teeth in a bucco-lingual direction. The slices were dehydrated and embedded in paraffin. Contact microradiographs were then made on Kodak high-resolution plates[20].

For the purpose of studying the normal vascular system of the pulp and as a control of the laboratory procedures 1 mm thick slices of 20 clinically healthy premolars, one from each quadrant of the jaws, were microradiographed. These control teeth were not exposed to any experimental procedure.

RESULTS

Control teeth

Blood vessels containing contrast medium were seen in the whole pulp. The larger vessels were centrally located and ran mainly in a longitudinal direction from the apical foramina to the coronal pulp or vice versa. These vessels were surrounded by some intermediate-sized blood vessels giving off branches that terminated in a subdentinal

capillary plexus which was most pronounced in the coronal pulp (Fig. 2a).

Replanted and autotransplanted teeth with intact roots

Ten days postoperatively (eight teeth). In the pulp of six teeth blood vessels were not observed (Fig. 2b). In the remaining two teeth a few slender vessels were present in the apical area (Table II).

Thirty days postoperatively (nine teeth). The pulp of seven teeth was completely void of contrast-filled blood vessels. In the remaining two teeth vessels with a few branches were found apically (Fig. 3a, Table II).

One hundred and twenty days postoperatively (nine teeth). Blood vessels were not detected in the pulp of any of the teeth (Figs. 4a, 5a and 6a, Table II).

Replanted and autotransplanted teeth with apicoectomized roots

Ten days postoperatively (eight teeth). In three teeth blood vessels were not observed in the pulp. In the remaining five the apical part of the pulp showed contrast-filled vessels which ran parallell to each other and the dentinal walls giving off numerous branches (Fig. 2c, Table III).

Thirty days postoperatively (nine teeth). The pulp of one tooth was completely devoid of contrast-filled blood vessels. Four teeth exhibited a great number of vessels giving off branches, but the area that was vascularized did not exceed three fourths of the length of the pulp (Table III). In the remaining four teeth blood vessels filled with contrast medium were present in all areas (Fig. 3b, Table III).

One hundred and twenty days postoperatively (nine teeth). In one tooth blood vessels of various sizes were found only in the apical region of the pulp (Fig. 6b). In the remaining eight teeth the whole pulp showed contrastfilled blood vessels (Figs. 4b, 5b and 5c). In one of these teeth poor filling of the contrast medium was noted in the coronal vessels (Fig. 4b). In all teeth numerous branches were observed and probably also anastomoses between vessels, but a subdentinal capillary plexus was not seen (Figs. 4b, 5b, 5c and 6b, Table III).

DISCUSSION

The microangiographic technique in this investigation has been discussed in detail in a previous paper[20]. With this technique it is possible to achieve a good picture of the microvascular pattern of the pulp, and even extremely thin branches can be visualized[20]. In the control teeth in the present study blood vessels with branches and slender ramifications, terminating in a subdentinal capillary plexus, were seen in the entire pulp. The arrangement of the vascular tree resembled that of human teeth[21].

The results showed that pulpal revascularization did not occur in the teeth, which were replanted and autotransplanted with intact roots. This finding corresponded well with the results of other studies on adult dogs[4,11] and monkeys[13,16,22] and with findings in an investigation on autotransplanted mature human teeth[14].

In the present investigation apicoectomy was performed at the time of replantation or autotransplantation on some of the dog teeth coronally to the delta formed apical portion of the root canal. With this technique the area of the pulp exposed to the periapical tissues was markedly enlarged, which should facilitate pulpal revascularization. A gentle handling of the pulp tissue is, however, also essential. Preparation of a wider apical

foramen by means of burs, for example, has not been successful[23]. In the present study the apicoectomy was carried out by means of a sharp instrument causing a bleeding incisional wound in the pulp tissue. Great care was then taken not to traumatize this tissue further. In contrast to the nonapicoectomized teeth a majority of the apicoectomized teeth revascularized during a 120-day period. However, the normal vascular system of the pulp was not restored, especially not in the subdentinal areas. The revascularization rate was slower than in immature teeth[20], but the larger space between the apicoectomized tooth and the alveolar socket, might at least in part explain this difference.

Previously, only a few experimental investigations on mature replanted and autotransplanted apicoectomized teeth have been carried out, and mainly with histologic techniques[11,23]. Thus, Kosnevish[11] found pulpal repair in dogs 90-180 days postoperatively in 11 out of 36 apicoectomized teeth and only in exceptional cases in their contralaterals, which had not been apicoectomized. In clinical investigations varying results have been reported[2,12,15]. However, in these studies the root tip in some teeth has been accidentally, and in other teeth deliberately, fractured during the operative procedures, and differences in methods might at least in part explain the different results obtained.

In conclusion, mature replanted and autotransplanted teeth with about one fourth of the length of the root removed at the time of operation showed pulpal revascularization. The vessels had reached the pulp horn 120 days postoperatively in eight out of nine teeth. Mature teeth without apicoectomized roots did not revascularize. The effect of the apicoectomy technique on the revascularization capacity of the pulp was thus encouraging, and further studies should be carried out to elucidate the repair process of the pulp more in detail and also to assess the clinical application of the technique.

Table I. Distribution of the experimental material according to observation periods.

	No. of teeth	10 days	30 days	120 days
Replanted teeth	32	8	12	12
Transplanted teeth	20	8	6	6
Total	52	16	18	18

Table II. Area of pulp containing vessels filled with contrast medium. Nonapicoectomized teeth.

Observation periods (days)	No. of teeth	Area of pulp with visible vessels			
		0-1/4	1/4-2/4	2/4-3/4	3/4-4/4
10	8	8	0	0	0
30	9	9	0	0	0
120	9	9	0	0	0

Table III. Area of the pulp containing vessels filled with contrast medium. Apicoectomized teeth.

Observation periods (days)	No. of teeth	Area of pulp with visible blood vessels			
		0-1/4	1/4-2/4	2/4-3/4	3/4-4/4
10	8	7	1	0	0
30	9	3	1	1	4
120	9	1			8

LEGENDS

Fig. 1. Radiographs of maxillary first premolar (a), maxillary incisors (b), mandibular first premolar (c), and mandibular incisors (d) at the time of the operation. The root development is completed.

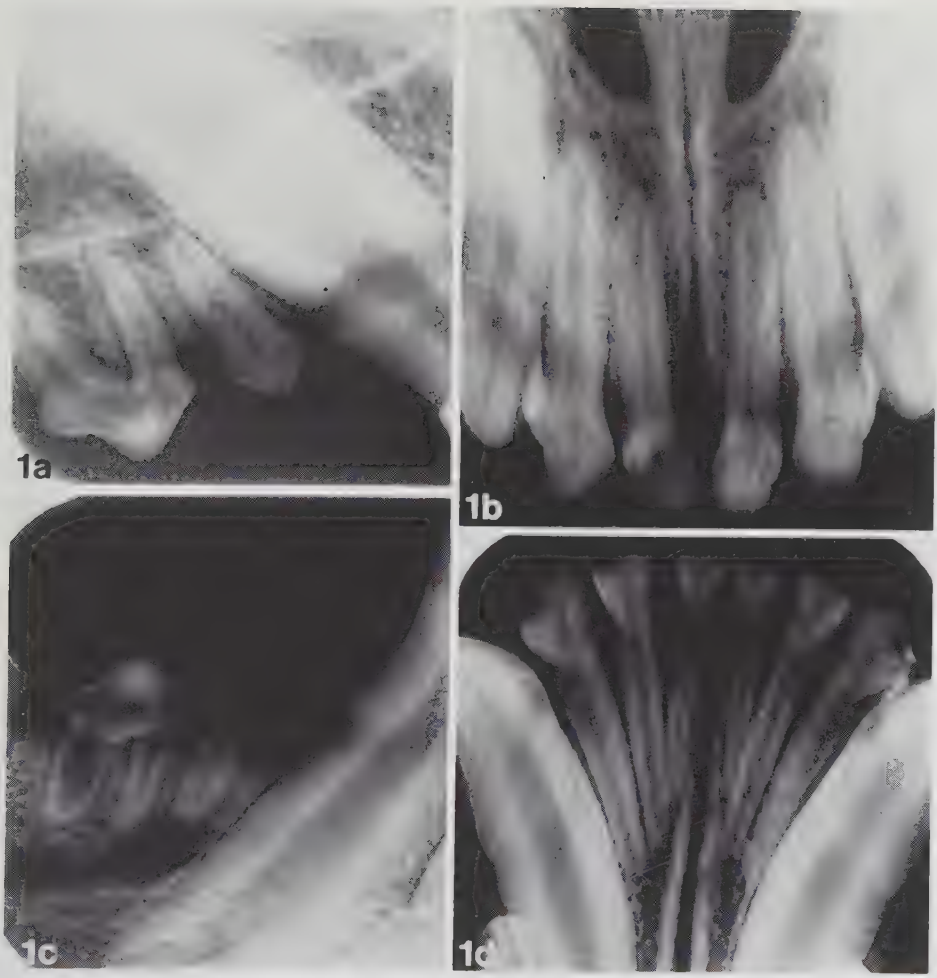

1a
1b
1c
1d

Fig. 2a. Microangiograph of a control tooth (mandibular second premolar). A bundle of central blood vessels ascends the radicular and coronal portion of the pulp, giving off numerous smaller branches that pass radially to terminate in a subdentinal capillary plexus. This plexus is especially pronounced in the coronal area of the pulp. A, Apical region. CD, Coronal dentin. P, Pulp. PM, Periodontal membrane. RD, Root dentin. (Magnification, x 10.)

Fig. 2b. Microangiograph of autotransplanted nonapicoectomized mandibular third incisor, 10 days postoperatively. Blood vessels are not observed in the pulp. A, Apical region. CD, Coronal dentin. P, Pulp. PM, Periodontal membrane. RD, Root dentin. (Magnification, x 10.)

Fig. 2c. Microangiograph of replanted apicoectomized maxillary second incisor, 10 days postoperatively. Numerous blood vessels with branches are seen in the apical portion of the pulp. These vessels run mostly parallel to each other and the dentinal walls. A, Apical region. CD, Coronal dentin. P, Pulp. PM, Periodontal membrane. RD, Root dentin. (Magnification, x 10.)

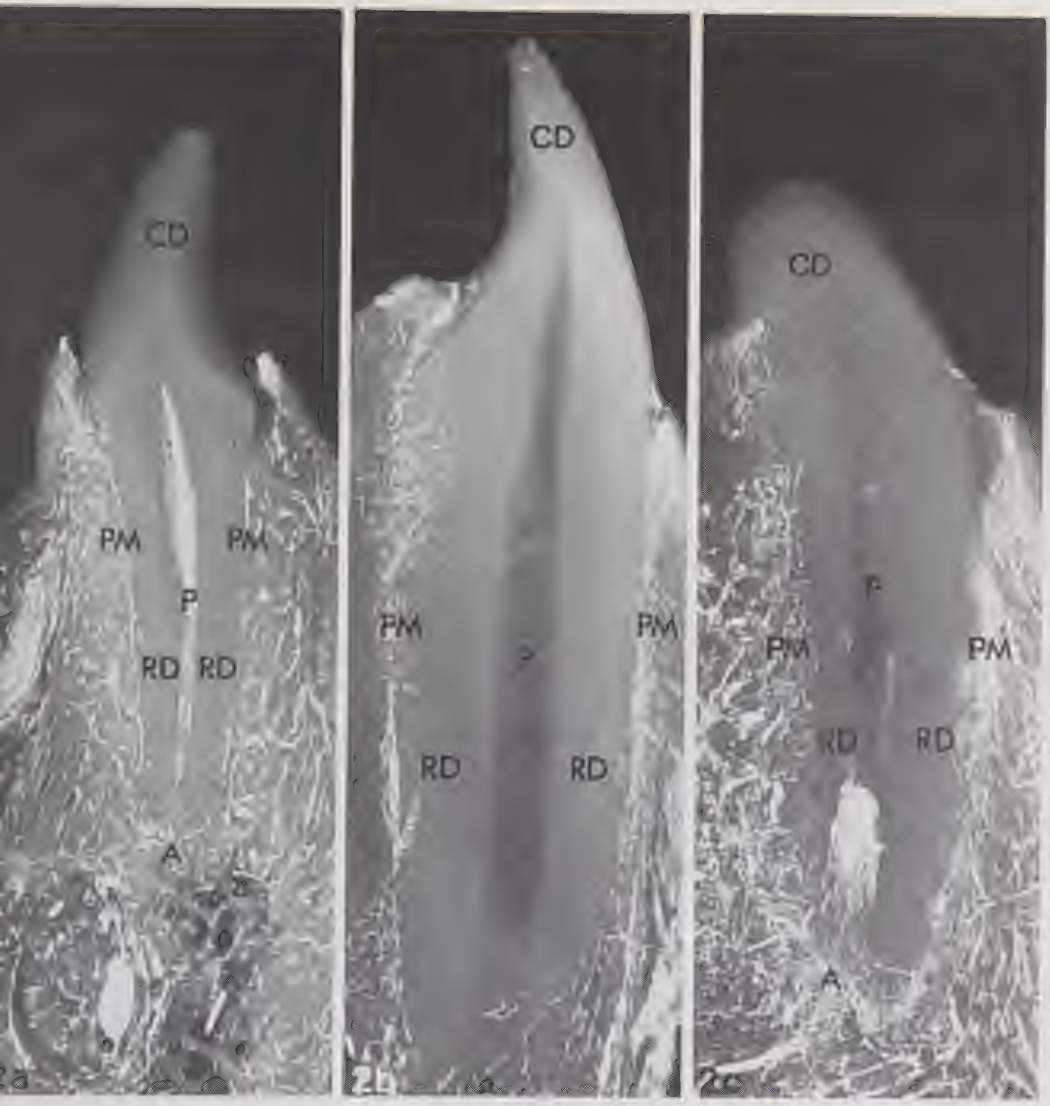

CD
PM
PM
P
RD
RD
A
CD
PM
PM
P
RD
RD
CD
P
PM
PM
RD
RD
A

Fig. 3a. Microangiograph of autotransplanted nonapicoectomized first premolar, 30 days postoperatively. Some blood vessels are found in the apical area of the pulp, running parallel to each other and the dentinal walls. A, Apical region. CD, Coronal dentin. P, Pulp. PM, Periodontal membrane. RD, Root dentin. (Magnification, x 10.)

Fig. 3b. Microangiograph of autotransplanted apicoectomized first premolar (contralateral to the tooth shown in Fig. 5a), 30 days postoperatively. Numerous vessels are seen running throughout the entire length of the pulp. Branches and apparently also anastomoses between vessels are also observed. A, Apical region. CD, Coronal dentin. P, Pulp. PM, Periodontal membrane. RD, Root dentin. (Magnification, x 10.)

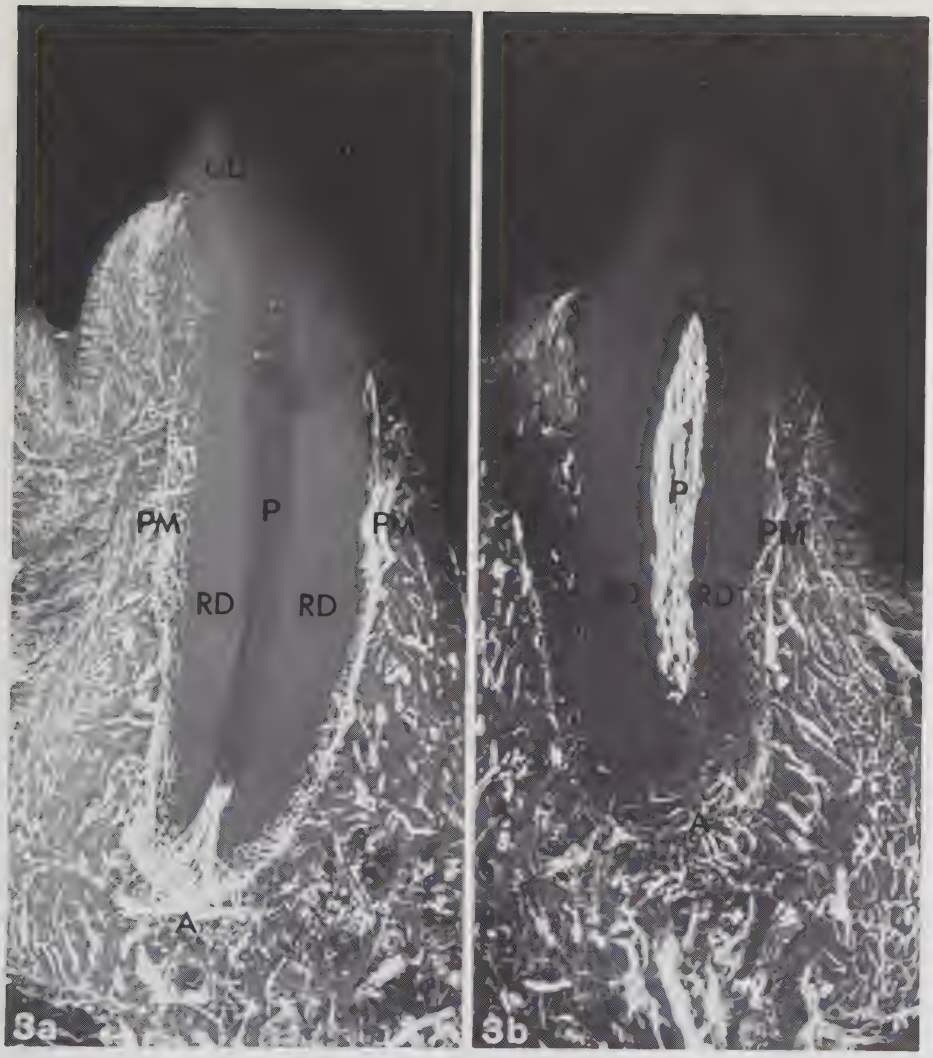

PM
P
PM
RD
RD
A
3a
P
PM
RD
A
3b

Fig. 4a. Microangiograph of autotransplanted nonapicoectomized mandibular third incisor, 120 days postoperatively. The pulp is completely devoid of visible blood vessels. A, Apical region. CD, Coronal dentin. P, Pulp. PM, Periodontal membrane. RD, Root dentin. (Magnification, x 10.)

Fig. 4b. Microangiograph of autotransplanted apicoectomized mandibular third incisor (contralateral to the tooth shown in Fig. 6a), 120 days postoperatively. Blood vessels with branches are seen in the entire root pulp. A few vessels are also found in the coronal pulp but are poorly filled by the contrast medium. A, Apical region. CD, Coronal dentin. P, Pulp. PM, Periodontal membrane. RD, Root dentin. (Magnification, x 10.)

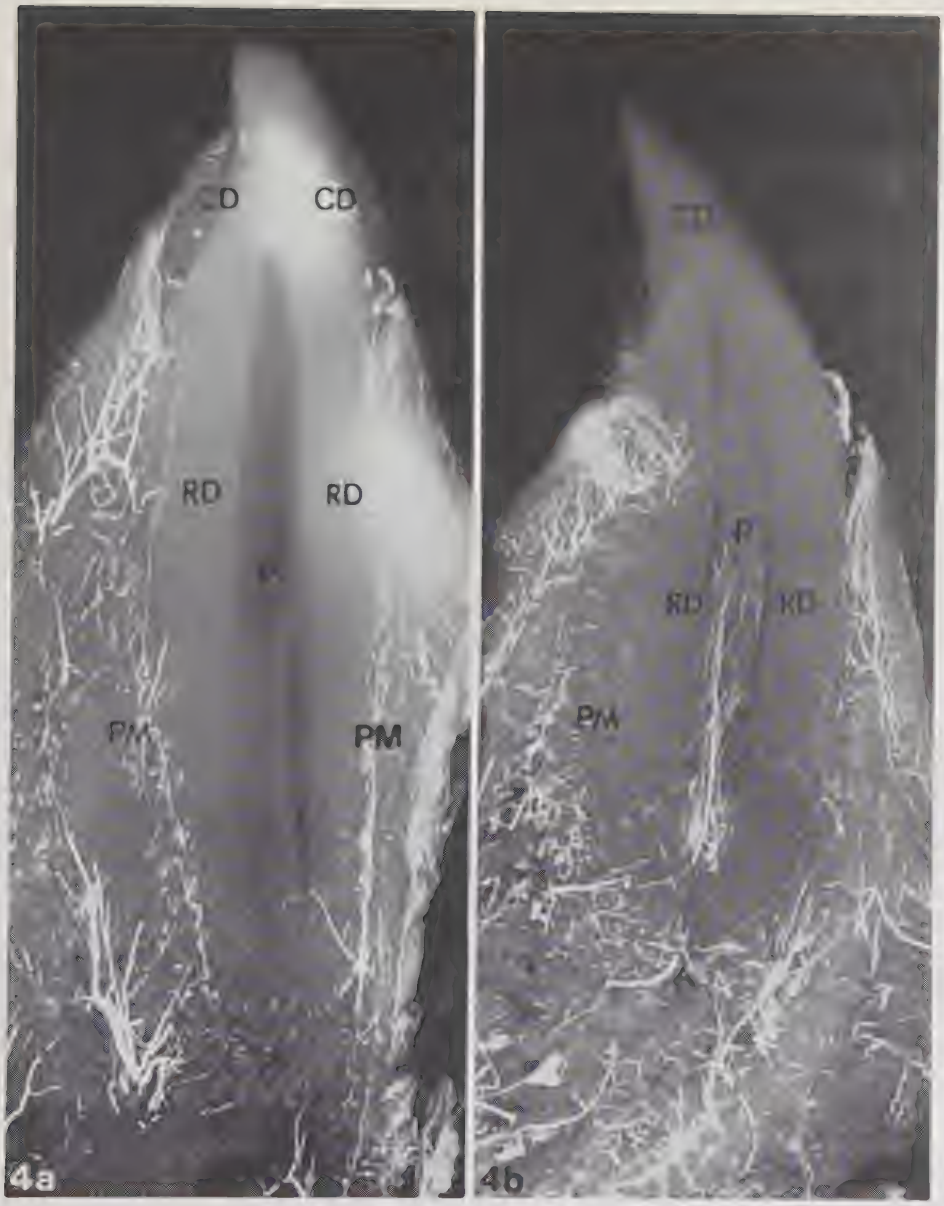

CD
CD
RD
RD
PM
PM
4a
RD
RD
PM
4b

Fig. 5a. Microangiograph of replanted nonapicoectomized maxillary third incisor, 120 days postoperatively. Blood vessels are not detected in the pulp. A, Apical region. CD, Coronal dentin. P, Pulp. PM, Periodontal membrane. RD, Root dentin. (Magnification, x 10.)

Fig. 5b. Microangiograph of replanted apicoectomized maxillary third incisor (contralateral to the tooth shown in Fig. 7a), 120 days postoperatively. Micropaque-filled blood vessels are observed throughout the pulp, the main vessels giving off numerous branches. A, Apical region. CD, Coronal dentin. P, Pulp. PM, Periodontal membrane. RD, Root dentin. (Magnifiaction, x 15.)

Fig. 5c. Microangiograph of autotransplanted apicoectomized mandibular second incisor, 120 days postoperatively. Blood vessels with branches are seen in the whole pulp. Note the difference between this tooth and the tooth shown in Fig. 5b as regards the width of the pulp cavity. A, Apical region, CD, Coronal dentin. P, Pulp. PM, Periodontal membrane. RD, Root dentin. (Magnification, x 10.

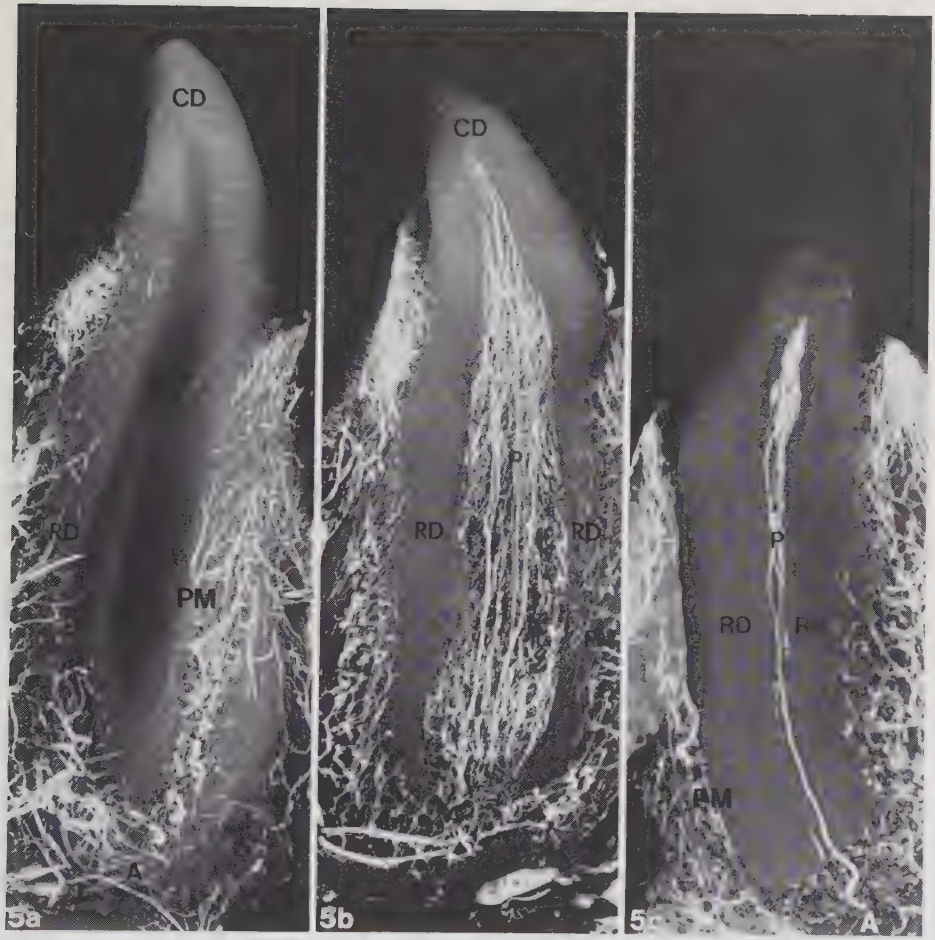
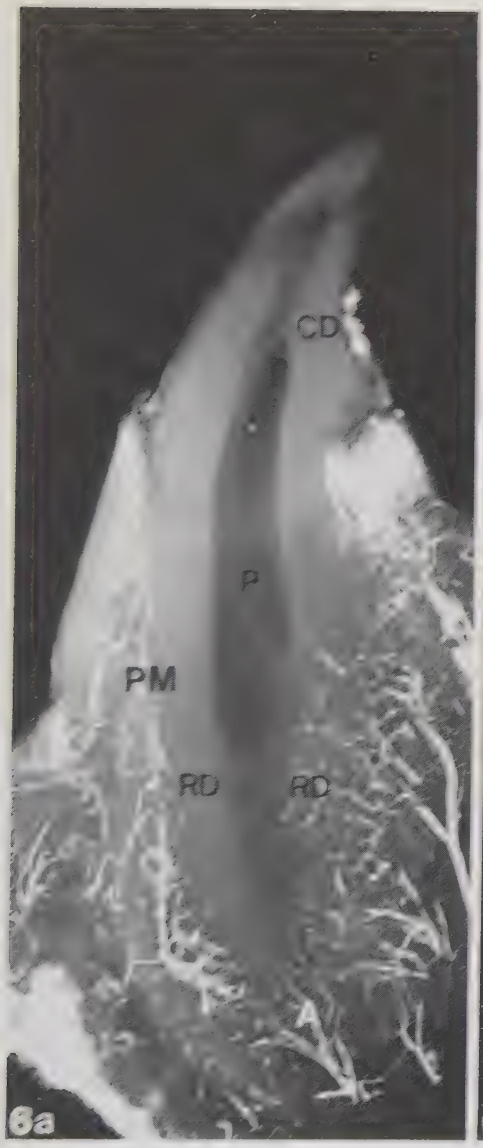
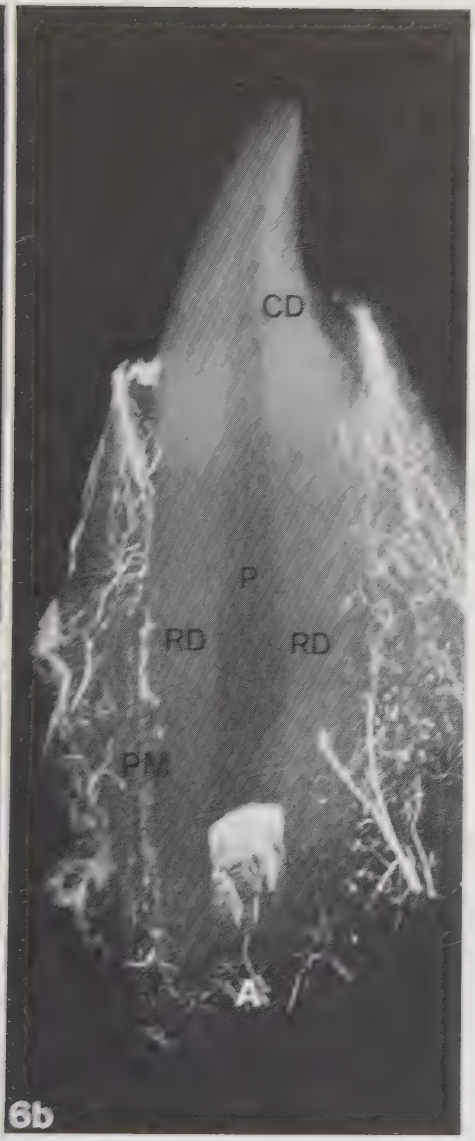

CD
RD
PM
A
5a
CD
P
RD
RD
5b
P
RD
PM
A

Fig. 6a. Microangiograph of autotransplanted nonapicoectomized maxillary second incisor, 120 days postoperatively. Blood vessels are not seen in the pulp. A, Apical region. CD, Coronal dentin. P, Pulp. PM, Periodontal membrane. RD, Root dentin. (Magnification, x 10.)

Fig. 6b. Microangiograph of autotransplanted apicoectomized maxillary second incisor (contralateral to the tooth shown in Fig. 8a), 120 days postoperatively. Micropaque-filled blood vessels of various diameter are found in the apical region of the pulp only. Note the width of the pulp cavity in this tooth as compared with the teeth shown in Figs. 5b and c. A, Apical region. CD, Coronal dentin. P, Pulp. PM, Periodontal membrane. RD, Root dentin. (Magnification, x 10.)



6a



6b

REFERENCES

1. WIDMAN, L.: Om transplantation av retinerade hörntänder. Sven, Tandlak. Tidsk. 1915:8:131-160.

2. FLATH, J.: Klinische beobachtungen bei der echten transplantation von retinierten zähnen. Dtsch. Stomat. 1963:13:561-574.

3. NORDENRAM, Å.: Autotransplantation of teeth. A clinical and experimental investigation. Acta Odontol. Scand. 1963:21: Suppl. 33.

4. KNIGHT, M.K., GANS, B.J. & CALANDRA, J.C.: The effect of root canal therapy on replanted teeth of dogs. A gross, roentgenographic and histologic study. Oral Surg. 1964:18:227-242.

5. ANDREASEN, J.O. & HJØRTING-HANSEN, E.: Replantation of teeth. I. Radiographic and clinical study of 110 human teeth replanted after accidental loss. Acta Odontol. Scand. 1966:24:263-286.

6. MOSS, J.P.: Autogenous transplantation of maxillary canines. J. Oral Surg. 1968:26:775-783.

7. NORDENRAM, Å.: Autotransplantation of teeth. Br. J. Oral Surg. 1969-1970:7:188-195.

8. THONNER, K.E. & MEIJER, M.: Autotransplantation of impacted upper canines. A clinical and histologic investigation. Odont. T. 1969;113-119.

9. THONNER, K.E. Autogenous transplantation of unerupted maxillary canines: A clinical and histologic investigation over five years. Dent. Practit. dent. Rec. 1971:21:251-257.

10. COOK, R.M.: The current status of autogenous transplantation as applied to the maxillary canine. Int. Dent. J. 1972:22:286-300.

11. KOSNEVICH, M.Y.: The state of the pulp in auto-transplantation of living teeth in an experiment with resection and without resection of the top of the root. Stomatol. (Mosc.) 1974:53 (2):1-4.

12. OKSALA, E.: Autotransplantation of vital maxillary canines. A clinical and radiographic study. Proc. Finn. Dent. Soc. 1974:70: Suppl. 1.

13. BARBAKOW, F.H., AUSTIN, J.C. & CLEATON-JONES, P.E.: Experimental replantation of root canal filled and untreated teeth in the vervet monkey. J. Endod. 1977: 3:89-93.

14. HASSELGREN, G., LARSSON, Å. & RUNDQUIST, L.: Pulpal status after autogenous transplantation of fully developed maxillary canines. Oral Surg. 1977:44: 106-112.

15. KALLIONEMI, H. & OKSALA, E.: Significance of an open apex or fracture of the root tip for the prognosis of vital maxillary canine autotransplantation. Proc. Finn. Dent. Soc. 1977:73:126-132.

16. NASJLETI, C.E., CAFFESE, R.G. & CASTELLI, W.A.: Replantation and autotransplantation of mature teeth without endodontics in monkeys. J. Dent. Res. 1978: 54:650-658.

17. HOVINGA, J.: Autotransplantation of maxillary canines: a long-term evaluation. J. Oral Surg. 1969:27:701-708.

18. ANDREASEN, J.O.: Traumatic Injuries of the Teeth. Munksgaard, Copenhagen, Denmark 1972: p. 212.

19. HEITHERSAY, G.S.: Replantation of avulsed teeth. A Review. Aust. Dent. J. 1975:20:63-72.

20. SKOGLUND, A., TRONSTAD, L. & WALLENIUS, K.: A micro-angiographic study of vascular changes in replanted and autotransplanted teeth of young dogs. Oral Surg. 1978:45:17-28.

21. SAUNDERS, R.L. de C.H.: Microradiographic studies of human adult and fetal dental pulp vessels. In Cosslett, V.E., Engström, A., and Pattee, H.H., editors: X-Ray Microscopy and Microradiography, New York, 1957, Academic Press, Inc., pp. 561-571.

22. SKOGLUND, A.: Autotransplantation of fully developed monkey teeth. To be published.

23. WALSH, J.S., KAFRAWY, A. & ROCHE, J.R.: The effect of apical modification on the vitality of replanted permanent monkey teeth. J. Dent. Child. 1978:45: 146-150.

V

PULPAL CHANGES IN REPLANTED AND AUTOTRANSPLANTED APICOECTOMIZED MATURE TEETH OF DOGS

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Running title: Pulpal changes in mature transplanted dog teeth.

Key words: apicoectomy, pulpal healing, root resorption, tooth reimplantation, tooth transplantation.

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ABSTRACT

The purpose of the present investigation was to study whether apicoectomy, performed at the time of the operation, affects the tissue reactions of the pulp of replanted and autotransplanted mature teeth. The material consisted of 45 teeth from six adult dogs. Twenty-six teeth were replanted or autotransplanted after removal of about one fourth of the length of the root. Nineteen teeth were replanted or autotransplanted without apicoectomy. The observation periods were 10, 30 and 120 days. The teeth were longitudinally sectioned at 7 µm in a bucco-lingual direction. The sections were stained with hematoxylin and eosin or according to the van Gieson or Brown and Brenn methods. No difference was noted in the tissue reactions of the pulp of replanted and autotransplanted teeth. In the apicoectomized teeth the pulp initially became necrotic, but repair occurred through ingrowth of connective that had reached the pulp horn in 14 of 15 teeth after 120 days. Cell-containing hard tissue had formed postoperatively on the root canal walls and in some teeth also centrally in the pulp. In the 19 nonapicoectomized teeth the pulp necrotized and repair did not occur.

INTRODUCTION

Pulpal changes in replanted and autotransplanted teeth have been studied previously in a number of investigations[1-14]. In contrast to immature teeth, mature teeth have not shown pulpal repair, except on rare occasions[1-14]. Attempts have been made, therefore, to improve the repair potential of the pulp by enlarging the apical foramen before replantation or autotransplantation. Diverging results have been obtained, however, conceivably due to variations in the operative techniques[9,15-20]. So far the most encouraging results have been gained by apicoectomy performed with a wire cutter[20]. In eight out of nine such apicoectomized mature teeth of dogs pulpal revascularization

had occurred as demonstrated with microangiography[20]. However, the characteristic vascular pattern of intact teeth and especially the subdentinal capillary plexus was not restored.

The purpose of the present investigation was to further study the pulp reaction of apicoectomized replanted and autotransplanted mature teeth by means of histologic techniques.

MATERIAL AND METHODS

The experimental material consisted of 45 single-rooted teeth (incisors and first premolars) in six adult mongrel dogs. Thirty-two of these teeth had been used previously in a microangiographic study[20]. The apical foramina were narrow in all teeth, indicating that root development was completed. The dogs were anesthetized by intravenous administration of a barbiturate solution (Penthotal sodium®, Abbott, Brussels, Belgium), and the experimental teeth were extracted. Eighteen of these teeth were then replanted and eight were autotransplanted to the socket of the contralateral tooth. At the operation about one fourth of the length of the root of these 26 teeth was removed (Table I). The apicoectomy was carried out by means of a wire cutter. Ten of the remaining experimental teeth were replanted and nine were autotransplanted without apicoectomy (Table I). The extraoral time did not exceed 10 minutes and during this period the teeth were stored in sterile isotonic saline solution at room temperature. The replanted and autotransplanted teeth were not immobilized by means of fixation devices. The dogs were put on a diet of soft food for 2 weeks or until the time of sacrifice. Penicillin (Penicillin prokain Vet.®, Novo, Copenhagen, Denmark) was given intramuscularly the day before the operation and the following seven days (600.000 I.U. per day). The observation periods chosen were 10, 30 and 120 days (Table II).

At the end of the observation periods the dogs were anesthetized by intravenous administration of a barbiturate solution (Mebumal Vet. ®, ACO, Solna, Sweden). Angiography was then performed with a barium sulfate injection method, according to a method described earlier, and the dogs were sacrificed during this procedure[13]. The jaws were then excised, fixed in 4 percent neutral-buffered formaldehyde and demineralized in 5 percent nitric acid. Tissue blocks, each containing one tooth, were dehydrated and embedded in paraffin. Longitudinal serial 7 µm thick sections were made from the teeth in a bucco-lingual direction. The sections were stained with hematoxylin and eosin or according to the van Gieson or Brown and Brenn methods. The staining according to the Brown and Brenn method included also tissue sections containing bacteria. Two clinically healthy premolars from each dog served as controls. These control teeth were not exposed to any experimental procedure.

RESULTS

Control teeth

A highly vascular, normal pulpal tissue with an even predentin zone and a regular odontoblastic layer was observed (Fig. 1). Bacteria were not found in this tissue or in the dentinal tubules. The main root canal divided apically into many small channels containing soft tissue (Fig. 1). Internal or external root resorption lacunae were not seen.

Experimental teeth

Some of the teeth showed slight damage due to the extraction procedures and cracks with exposure of the dentin in the cervical areas were evident. A few bacteria were observed in the cracks and in plaque on the tooth surface. Also a few bacteria were found in necrotic pulp tissue in

one tooth. No difference was noted in the tissue reactions of the pulp of replanted and autotransplanted teeth.

Replanted and autotransplanted teeth with intact roots

Ten days postoperatively (three teeth). A weakly stained tissue with small roundish cell-nuclei and otherwise only vaguely discernible tissue structures was seen throughout the pulp (Fig. 2a). Small external root resorption lacunae were found cervically as well as in the middle and apical portion of the root. These lacunae were rich in small blood vessels, and multinucleated cells and a few inflammatory cells were observed.

Thirty days postoperatively (seven teeth). In one tooth a vascularized connective tissue with extravasated erythrocytes was evident in the apical area of the pulp. Further coronally in this tooth, as well as throughout the pulp of the remaining six teeth, a weakly stained or slightly eosinophilic tissue was seen, and the different tissue structures were hardly discernible. External root resorption lacunae were observed in all teeth and were usually somewhat larger than in the 10-day teeth. Adjacent to and inside the lacunae, numerous small blood vessels were present as well as multinucleated cells and a few inflammatory cells. Advanced external root resorption had occurred apically in one tooth and had resulted in a shortening of the length of the root. In this specimen accumulations of inflammatory cells were found in the periapical tissue.

One hundred and twenty days postoperatively (nine teeth). In one tooth vascularized connective tissue was seen in the apical part of the pulp, and cell-containing, atubular hard tissue had been laid down on the root canal walls. Further coronally in this tooth, as well as throughout the pulp of the remaining eight teeth (Fig. 2b), the different tissue structures were weakly stained and hardly or not at all discernible. Small, roundish cell-nuclei were,

however, seen in some teeth (Fig. 2b). External root resorption lacunae, which did not exceed one third of the thickness of the dentin were observed in all teeth. In six teeth some lacunae were filled with hard tissue while other lacunae were not. Some of the latter showed multinucleated cells and/or scattered inflammatory cells, but a heavy inflammatory reaction was not noted neither in the lacunae nor in the adjacent areas of the periodontal membrane and bone. In three teeth apposition of a cell-containing hard tissue was observed in all lacunae (Fig. 2c), although pulpal repair had not occurred. Accumulations of a few baceria were found in the root pulp in one of these teeth.

Replanted and autotransplanted teeth with intact roots

Ten days postoperatively (six teeth). In five teeth a cell-rich, well-vascularized and well-stained connective tissue was found in the apical region of the pulp (Fig. 3). Odontoblasts were not observed in this area. Further coronally in these five teeth, and in the entire pulp of the remaining tooth, an avascular weakly stained tissue with small roundish cell-nuclei was seen. Accumulations of extravasated erythrocytes and/or inflammatory cells were found in the border area between vascularized and nonvascularized tissue (Fig. 3). In one tooth internal root resorption lacunae were evident in the apical area of the pulp (Fig. 3). The lacunae were rich in blood vessels, and showed a few multinucleated cells and a few inflammatory cells.

Thirty days postoperatively (five teeth). A well-vascularized, cell-rich connective tissue was seen in the apical area of the pulp of one tooth and throughout approximately half of the length of the pulp of three teeth. Further coronally in these four teeth a weakly stained, avascular tissue was observed, and the different tissue structures were hardly discernible. In the border area between vascularized and nonvascularized tissue small

accumulations of extravasated erythrocytes and/or inflammatory cells were noted. The remaining tooth showed a vascularized connective tissue in the whole pulp cavity. In this tooth cell-containing, atubular hard tissue had formed on the root canal walls and centrally in the pulp. Odontoblasts were not found in any of the teeth. Small apical and lateral external root resorption lacunae were seen in all teeth. Some lacunae showed apposition of a cellular hard tissue and in other lacunae multinucleated cells and a few inflammatory cells were observed.

One hundred and twenty days postoperatively (Fifteen teeth). In 14 teeth a vascularized, well-stained connective tissue was observed throughout the pulp (Figs. 4a, 4b and 5a). Areas of structureless tissue were found in the central part of the coronal pulp in four of these teeth (Fig. 5a). Small accumulations of extravasated erythrocytes and inflammatory cells were seen in these and another two teeth. Macrophages were scattered throughout the pulp in a majority of the specimens. Cell-containing, atubular hard tissue had formed on the root canal walls in all teeth and centrally in six teeth (Fig. 5b). Small internal resorption lacunae were found in nine teeth. Apposition of hard tissue was noted in these lacunae in a majority of the teeth. Apical and lateral external root resorption lacunae were observed in all teeth (Figs. 4a, 5a and 5c). The resorption process had not penetrated through more than one third of the thickness of the dentin in all but one tooth, in which the process had reached the pulp cavity in the cervical area (Fig. 5b). In eight teeth all lacunae showed apposition of cell-containing hard tissue (Fig. 4a). In the remaining teeth a few inflammatory cells were seen in some of the lacunae, and in these lacunae apposition of hard tissue was not found (Fig. 5c).

In one tooth connective tissue with contrast-filled blood vessels was observed in the apical area of the pulp (Fig. 4c). In this area large internal root resorption lacunae with and without apposition of hard tissue was seen. Further coronally, a weakly stained, structureless nonvascularized tissue was found. In the border zone between vascularized and nonvascularized tissue large accumulations of extravasated erythrocytes and inflammatory cells were observed.

DISCUSSION

The results of this investigation showed that the pulp of the mature nonapicoectomized replanted and autotransplanted teeth necrotized, and that repair did not occur. This finding corresponded well with the results of previous experimental studies on adult dogs[5,9,20], and monkeys[10,12,21], and also with findings in mature autotransplanted human teeth[11].

It has, however, been shown with microangiography[20], that the revascularization capacity of the pulp of mature replanted and autotransplanted teeth can be markedly improved if apicoectomy is carried out at the time of the operation. In the present investigation it was possible to obtain a more detailed picture of the effect of apicoectomy on the tissue reactions of the pulp by means of histologic techniques.

In the apicoectomized replanted and autotransplanted teeth in the present study pulpal repair was evident. The repair process was similar to that shown in immature teeth[22] but proceeded in a slower rate. Apparently the entire pulp first became necrotic and then underwent repair through proliferation and migration of cells from the recipient bed, i.e. the periapical region.

The connective tissue formed in the pulpal cavity was later in part replaced by a bone- or cementum-like hard

tissue. Pulpal obliteration has been interpreted as a sign of pulpal vitality[3,4,23,24,25], and has been considered a positive reaction after autotransplantation of teeth[3,23]. It should be borne in mind, however, that obliterated teeth might necrotize[26], and that endodontic treatment in such teeth is complicated or even infeasible. It would, therefore, be a great advantage if survival of the original pulpal tissue could be achieved, since such changes as obliteration and also internal resorption then do not seem to occur[22]. A prerequisite for pulpal survival is a rapid revascularization with the formation of anastomoses between vessels from the periapical tissue and vessels from the pulp[13,14]. However, such rapid revascularization should apparently not be expected in mature apicoectomized transplants[20].

A massive early infection during the postoperative period is known to interfere with the repair of the periodontal membrane, and may lead to a rapid loss of the autotransplanted tooth[27,28]. A noninfectious recipient bed is probably also a prerequisite for pulpal repair, and in the present investigation it was interesting to note that no stainable bacteria were present in the pulp cavity in the apicoectomized teeth. However, the antiseptic conditions during the operation and the use of antibiotics minimized the risk of infection and most probably contributed to the results obtained.

It has been shown in previous studies[29-31] that sterile necrotic tissue gives rise to only a slight inflammatory reaction in the organism, and the results in the present investigation coincide with these findings. Large accumulations of inflammatory cells were seen in only one apicoectomized tooth that showed a retarded pulpal repair 120 days postoperatively, and in this tooth advanced internal resorption was observed.

It has been suggested that necrotic pulp tissue may give rise to external rooth resorption of the inflammatory

type[32,33]. This view is supported by the finding that proper endodontic treatment may lead to arrest of the resorption process[34,35].

External root resorption affected all replanted and autotransplanted teeth in the present study. However, repair of most of the resorption cavities was seen 120 days postoperatively, both in the apicoectomized teeth, in which pulpal repair was observed, and in the nonapicoectomized teeth, that had necrotized and did not show pulpal repair. Thus, external inflammatory root resorption seems to be promoted by a number of concurrent factors. One such factor might be bacteria[33].

In conclusion according to the findings in the present study the pulp of the apicoectomized replanted and autotransplanted teeth first became necrotic and then underwent repair. The pulp of the nonapicoectomized teeth, on the other hand, necrotized, and repair did not occur. Before the apicoectomy technique is applied to the clinic, it should be borne in mind, however, that this study was performed on dogs, and that there might be differences between species as regards the repair capacity of the pulp. Further, possible clinical implications of pulpal obliteration should be considered and weighed against the disadvantages of endodontic treatment.

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Table I. Distribution of experimental material

	No. of teeth	First incisor	Second incisor	Third incisor	First premolar
Apicoectomized teeth					
Replanted teeth	18	2	5	6	5
Transplanted teeth	8	1	4		3
Nonapicoectomized teeth					
Replanted teeth	10		3	4	3
Transplanted teeth	9	3	3	1	2
Total	45	6	15	11	13

Table II. Distribution of experimental material according to observation periods

	No. of teeth	Observation periods		
		10 days	30 days	120 days
Apicoectomized teeth	26	6	5	15
Nonapicoectomized teeth	19	3	7	9
Total	45	9	12	24

LEGENDS

Fig. 1. Control tooth (mandibular second premolar). A normal pulpal tissue, an even predentin zone and a regular odontoblastic layer is observed. In the apical region the main root canal divides into many small channels containing soft tissue. A, Apical region. B, Bone. CD, Coronal dentin. P, Pulp. RD, Root dentin. (Hematoxylin and eosin stain. Magnification, x 10).

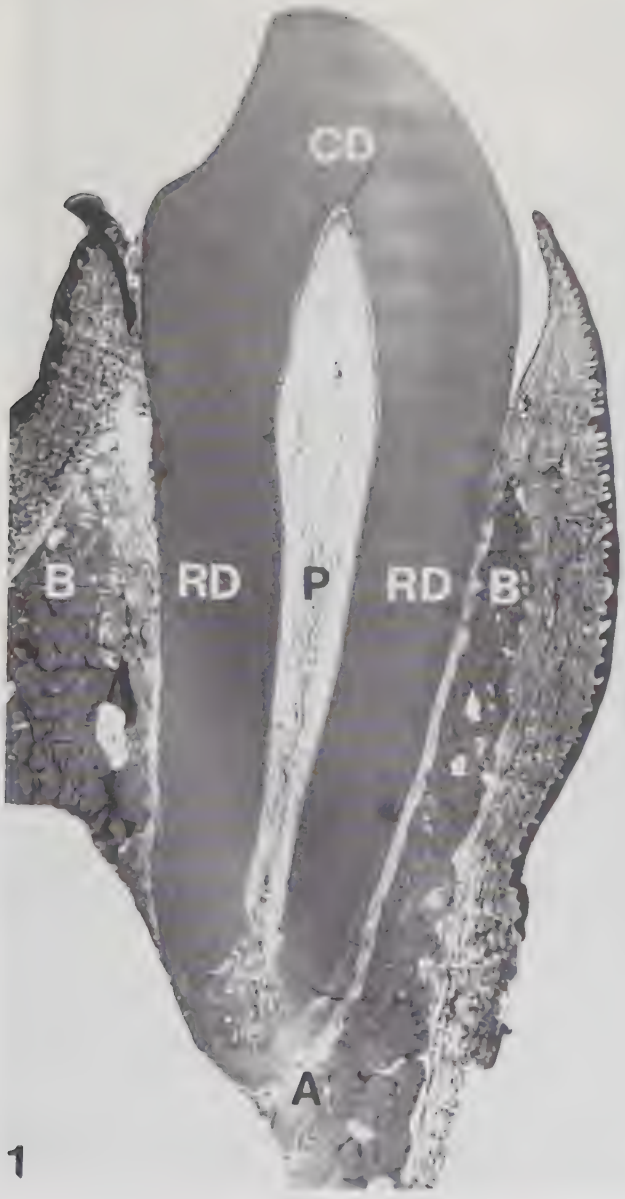

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Fig. 2a. Replanted nonapicoectomized maxillary second incisor, 10 days postoperatively. The connective tissue in the pulp including the odontoblastic layer is weakly stained and contrast-filled blood vessels are not observed. A, Apical area. CD, Coronal dentin. P, Pulp. RD, Root dentin. (Hematoxylin and eosin stain. Magnification, x 10).

Fig. 2b. Replanted nonapicoectomized mandibular first premolar, 120 days postoperatively, middle portion of the pulp. A weakly stained tissue with roundish cell-nuclei is seen. The odontoblastic layer is marked with arrows. (Hematoxylin and eosin stain. Magnification, x 400).

Fig. 2c. Autotransplanted nonapicoectomized maxillary second incisor, 120 days postoperatively, apical one third of the root. External root resorption lacunae with apposition of a cell-containing hard tissue is noted (at arrows). A structureless tissue is observed in the pulp cavity (to the right). RD, Root dentin. P, Pulp. (Magnification, x 100).

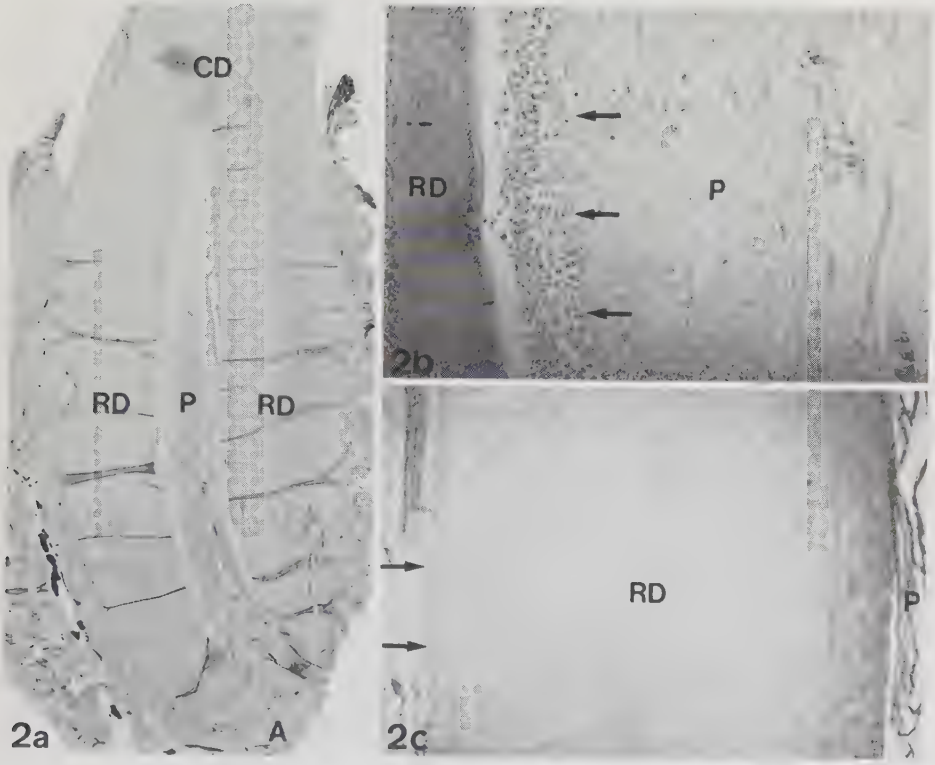

CD
RD
P
RD
A
2a
RD
P
2b
RD
P
2c

Fig. 3. Replanted apicoectomized mandibular incisor, 10 days postoperatively. A well-vascularized connective tissue is seen in the apical area of the pulp and contains numerous contrast-filled blood vessels (at arrows). Further coronally, a weakly stained tissue is evident, and the different tissue structures are only vaguely discerned. A, Apical area. P, Pulp. RD, Root dentin. (Hematoxylin and eosin stain. Magnification, x 60).

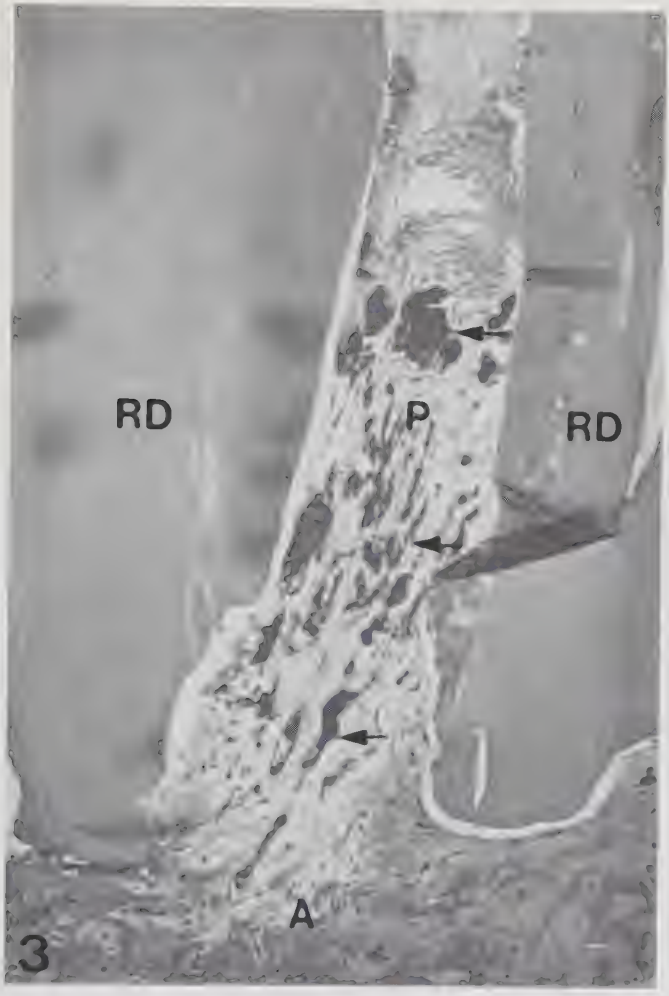

RD
P
RD
A
3

Fig. 4a. Autotransplanted apicoectomized maxillary first incisor, 120 days postoperatively. A well-stained, well-vascularized tissue is observed in the whole pulp cavity. A cell-containing hard tissue has formed on the root canal walls. External root resorption lacunae are seen in the cervical, middle and apical portion of the root (at arrows), and repair of the resorption cavities has occurred through apposition of hard tissue. A, Apical area. AF, Artifact. CD, Coronal dentin. P, Pulp. RD, Root dentin. (Hematoxylin and eosin stain. Magnification, x 10).

Fig. 4b. Detail from Fig. 4a, coronal area of the pulp. A well-stained cell-rich connective tissue is noted. CD, Coronal dentin. P, Pulp. (Magnification, x 400).

Fig. 4c. Autotransplanted, apicoectomized maxillary second incisor, 120 days postoperatively. A vascularized connective tissue is seen in the apical area of the pulp. Further coronally, a structureless avascular, weakly stained tissue is observed. In the border area between vascularized and nonvascularized tissue large accumulations of extravasated erythrocytes and inflammatory cells are found. Note the internal root resorption lacunae in the apical area. (Hematoxylin and eosin stain. Magnification, x 60).

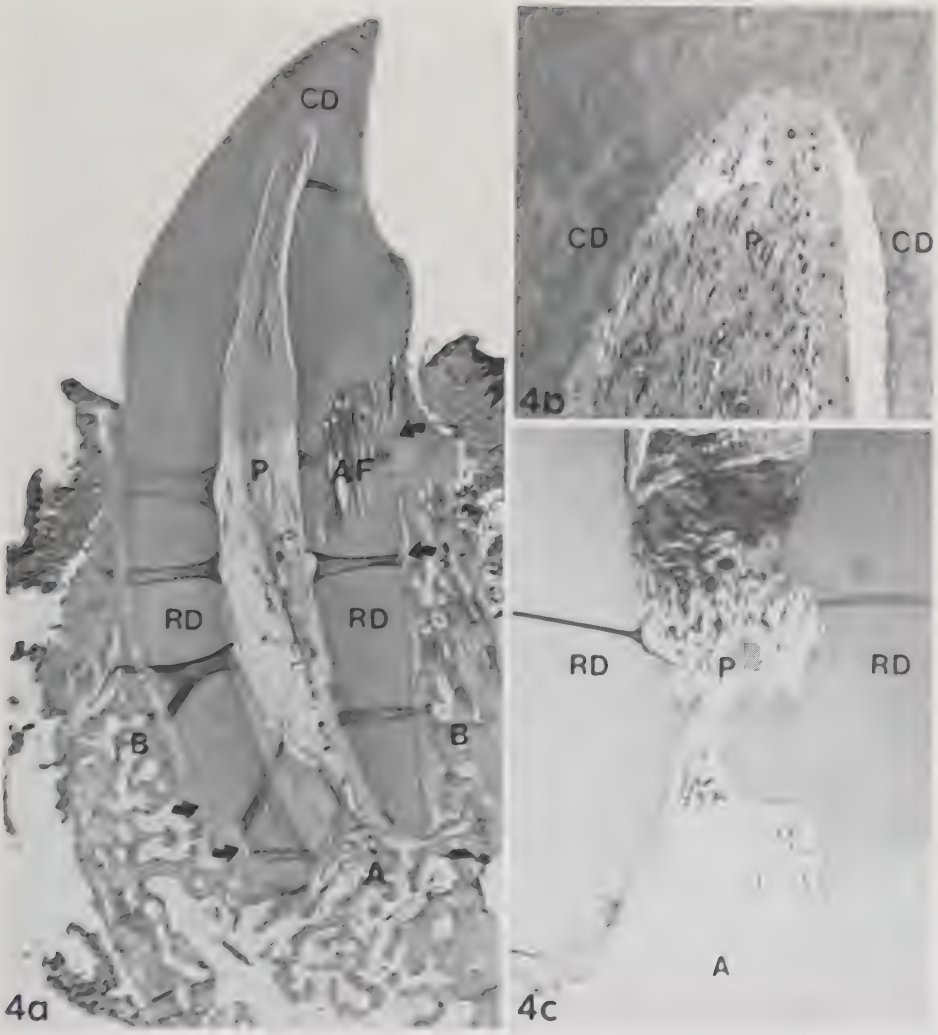
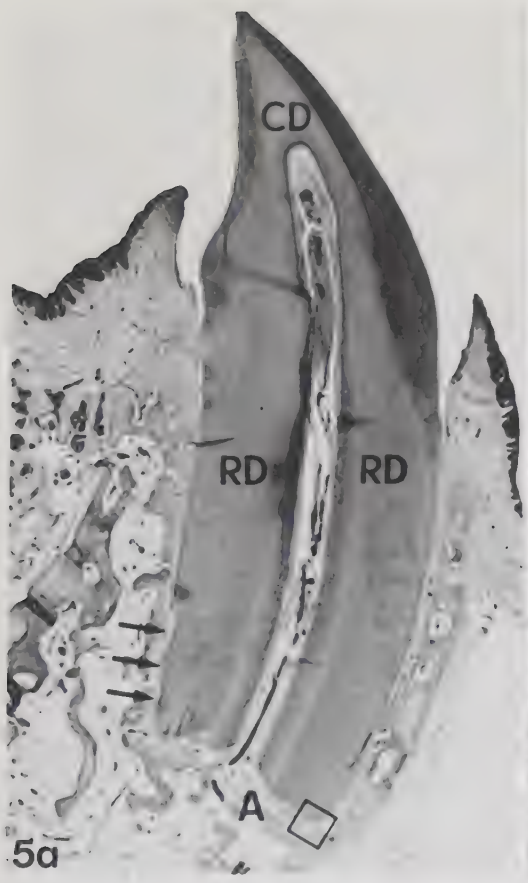

CD
P
AF
RD
RD
B
B
A
4a
CD
P
CD
4b
RD
P
RD
A
4c

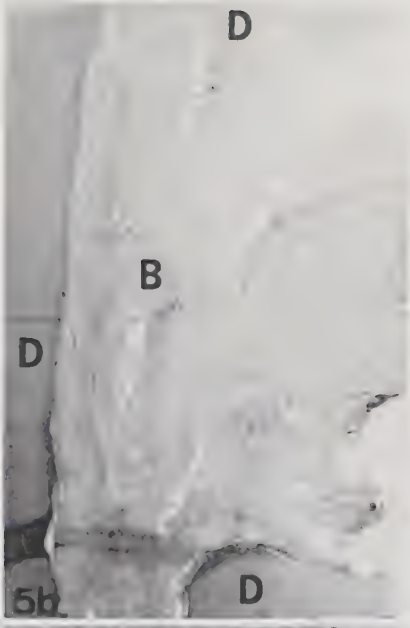
Fig. 5a. Replanted, apicoectomized maxillary first incisor, 120 days postoperatively. A vascularized connective tissue is observed throughout the pulp. In the central area of the coronal pulp a structureless tissue is noted. Only a small amount of hard tissue has formed postoperatively on the root canal walls. External root resorption lacunae with apposition of hard tissue are marked with arrows. A, Apical area. CD, Coronal dentin. RD, Root dentin. (Hematoxylin and eosin stain. Magnification, x 10).

Fig. 5b. Autotransplanted, apicoectomized maxillary first premolar, 120 days postoperatively. A large external root resorption cavity is noted cervically and the resorption process has reached the pulp cavity. A bone-like tissue (B) is seen in the central area of the pulp. D, Dentin. (Hematoxylin and eosin stain. Magnification, x 60).

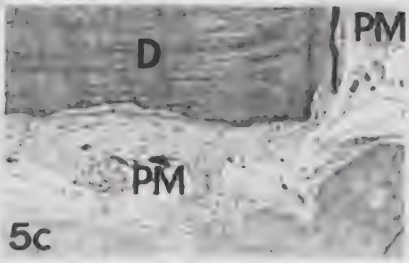
Fig. 5c. Detail from Fig. 5a, apical region of the root. External root resorption lacunae with and without apposition of hard tissue are observed. D, Dentin. PM, Periodontal membrane. (Magnification x 100).



5a



5b



5c

REFERENCES

1. WIDMAN, L.: Om transplantation av retinerade hörntänder. Sven. Tandlak. Tidskr. 1915:8:131-160.

2. AGNEW, R.G. & FONG, C.C.: Histologic studies on experimental transplantation of teeth. Oral Surg. 9: 18-39, 1956.

3. NORDENRAM, Å.: Autotransplantation of teeth. A clinical and experimental investigation. Acta Odontol. Scand. 1963:21:Suppl. 33.

4. ÖHMAN, A.: Healing and sensitivity to pain in young replanted human teeth. An experimental, clinical and histologic study. Odont. T. 1965:73:166-277.

5. KNIGHT, M.K., GANS, B.J. & CALANDRA, J.C.: The effect of root canal therapy on replanted teeth of dogs. A gross, roentgenographic and histologic study. Oral Surg. 1964:18:227-242.

6. THONNER, K.-E. & MEIJER, M.: Autotransplantation of impacted upper canines. A clinical and histologic investigation. Odont. T. 1969:77:113-119.

7. MONSOUR, F.N.T.: Pulpal changes following the reimplantation of teeth in dogs. Austr. Dent. J. 1971: 16:227-231.

8. THONNER, K.-E.: Autogenous transplantation of unerupted maxillary canines: A clinical and histologic investigation over five years. Dent. Practit. dent. Rec. 1971:21:251-257.

9. KOSNEVISH, M.Y.: The state of the pulp in autotransplantation of living teeth in an experiment with resection and without resection of the top of the root. Stomatol. (Mosc.) 1974:53:1-4.

10. BARBAKOW, F.H., AUSTIN, J.C. & CLEATON-JONES, P.E.: Experimental replantation of root canal-filled and untreated teeth in the vervet monkey. J. Endod. 1977:3:89-93.

11. HASSELGREN, G., LARSSON, Å. & RUNDQUIST, L.: Pulpal status after autotransplantation of fully developed maxillary canines. Oral Surg. 1977:44: 106-112.

12. NASJLETI, C.E., CAFFESE, R.G. & CASTELLI, W.A.: Replantation and autotransplantation of mature teeth without endodontics in monkeys. J. Dent. Res. 1978: 54:650-658.

13. SKOGLUND, A., TRONSTAD, L. & WALLENIUS, K.: A microangiographic study of vascular changes in replanted and autotransplanted teeth of young dogs. Oral Surg. 1978:45:17-28.

14. SKOGLUND, A., HASSELGREN, G. & TRONSTAD, L.: Oxidoreductase activity in the pulp of replanted and autotransplanted teeth in young dogs. Accepted for publication, Oral Surg.

15. FLATH, J.: Klinische beobachtungen bei der echten transplantation von retinierten zähnen. Dtsch. Stomat. 1963:13:561-574.

16. BOLTON, R.: Autogenous transplantation and replantation of teeth: Report on 60 treated patients. Br. J. Oral Surg. 1974:12:147-165.

17. OKSALA, E.: Autotransplantation of vital maxillary canines. A clinical and radiographic study. Proc. Finn. Dent. Soc. 1974:70: Suppl. I.

18. KALLIONEMI, H. & OKSALA, E.: Significance of an open apex or fracture of the root tip for the prognosis of vital maxillary canine autotransplantation. Proc. Finn. Dent. Soc. 1977:73:126-132.

19. WALSH, J.S., KAFRAWY, A. & ROCHE, R.R.: The effect of apical modification on the vitality of replanted permanent monkey teeth. J. Dent. Child. 1978:45:146-150.

20. SKOGLUND, A.: Vascular changes in replanted and autotransplanted mature teeth with apicoectomized roots. Submitted for publication, Int. J. Oral Surg.

21. SKOGLUND, A.: Autotransplantation of fully developed monkey teeth. To be published.

22. SKOGLUND, A. & TRONSTAD, L.: Pulpal changes in replanted and autotransplanted immature teeth of dogs. Submitted for publication, Oral Surg.

23. LOVIUS, B.B.J., ATHERTON, J.D., WYNNE, T.H.M. & FINCH, L.D.: Autogenous tooth transplantation: a clinical and histological investigation. Br. J. Orthod. 1972:1:27-33.

24. OKSALA, E. & KALLIONEMI, H.: A longitudinal clinical and radiographic study of autotransplantation of the maxillary canine. Proc. Finn. Dent. Soc. 1977:73:117-125.

25. SLAGSVOLD, O. & BJERCKE, B.: Autotransplantation av premolarer. Göteborgs Tandl. Sällskaps Årsb. 1967: no 351:45-85.

26. ANDREASEN, J.O., HJØRTING-HANSEN, E. & JØLST, O.: A clinical and radiographic study of 76 autotransplanted third molars. Scand. J. Dent. Res. 1970:78: 512-523.

27. SINGH, K.K. & DUDANI, I.C.: Autogenous transplantation of developing mandibular third molars. J. Indian Dent. Assoc. 1970:42:199-212.

28. MOSS, J.P.: The indications for the transplantation of maxillary canines in the light of 100 cases. Br. J. Oral Surg. 1975:12-268-274.

29. TORNECK, C.D.: Reaction of rat connective tissue to polyethylene tube implants. Oral Surg. 1967:24: 647-683.

30. BERGENHOLZ, G.: Microorganisms from necrotic pulp of traumatized teeth. Odont. Revy 1974:25:347-358.

31. MAKKES, D.C., TODEN van VELZEN, S.K. & WESSELINK, P.R.: The response of the living organism to dead and fixed dead tissue. J. Endod. 1978:4:17-21.

32. ANDREASEN, J.O. & HJØRTING-HANSEN, E.: Replantation of teeth. II. Histologic study of 22 replanted anterior teeth in humans. Acta Odontol. Scand. 1966:24:287-306.

33. ANDREASEN, J.O.: Traumatic Injuries of the Teeth. Munksgaards, Copenhagen, Denmark. 1972: pp. 180-182, 206-211.

34. ANDREASEN, J.O.: Treatment of fractured and avulsed teeth. J. Dent. Child. 1971:38:29-48.

35. ÇVEK, M.: Treatment of non-vital permanent incisors with calcium-hydroxide. II. Effects on external root resorption in luxated teeth compared with effect of root filling with guttapercha. A follow-up. Odont. revy 1973:24:343-354.